Project title:	An investigation of the current status of tomato leaf mould on UK nurseries: occurrence, disease management and potential for improved control
Project number:	PE 030
Project leader:	Sarah Mayne, RSK ADAS (January 2016 – December 2016) Dave Kaye, RSK ADAS (December 2017 – March 2017)
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Date project commenced:	1 April 2016

Date project completed	31 March 2017
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DISCLAIMER

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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GROWER SUMMARY

Headline

- Tomato leaf mould was present on at least six UK tomato nurseries and on 16 different varieties in 2016.
- Disease spread on varieties with no claimed resistance can be very rapid.
- Growers successfully manage the disease by a combination of measures: plant protection products; humidity control; hygiene; de-leafing hard; rotation and floor covering.

Background

Tomato leaf mould caused by *Passalora fulva* (previously *Cladosporium fulvum*) is a destructive foliar disease of increasing importance in the UK. New strains of the pathogen have recently been identified in Japan, China and Korea for which no varietal resistance is in place, the disease is also causing considerable problems in Argentina. Outbreaks have occurred most years over the last decade and affected a range of varieties (Figure 1). Previously well controlled by genetic resistance, the new outbreaks appear to be caused by the cultivation of varieties with no claimed resistance and the emergence of strains capable of overcoming the resistance genes deployed in current varieties. Currently there is no easy method to identify strains; the classical approach is to determine pathogenicity of isolates by inoculation on to a differential set of tomato varieties that possess different resistance genes. This involves testing one isolate at a time on varieties containing known resistance genes, which is both time-consuming and costly. Furthermore, identification of races 1 and 3 is not perfect in this system and no molecular methods to identify different strains have been developed to our knowledge.

Additionally, although Amistar (azoxystrobin) has given good control in some crops, grower reports indicate fungicide resistant strains can develop within a few years. Previous work, PE 018, showed a number of other products including Switch (cyprodinil + fludioxonil) and Signum (boscalid + pyraclastrobin) also give good control when used preventatively. The new product Prolectus (fenpyrazamine) warrants evaluation as the closely related fungicide Teldor (fenhexamid) gave some control in PE 018. Similarly, one or more succinate dehydrogenase inhibitor (SDHI) fungicides submitted for authorisation to use on protected tomato warrant laboratory evaluation as these are generally fungicides with broad spectrum activity.

The disease also affects organic crops, where use of conventional fungicides is not permitted by the Soil Association. Nursery sanitation and hygiene measures between crops, choice of variety and glasshouse environment control are critical in this situation. Spores of *P. fulva* appear to be very resistant to dryness and low temperatures, and are believed to survive in a dormant state from one crop to the next. The fungus can also survive saprophytically in dried leaf debris. A number of disinfectants were shown to be effective against *P. fulva* in PE 018, however effective crop clean up remains problematic on several commercial sites, with re-infection commonly occurring year on year.

Figure 1. Yellow spots on the upper leaf surface are an early symptom of tomato leaf mould infection.

Further effort to identify gaps/weaknesses in current control practices and establish improved targeted, integrated control measures could be beneficial, as incidence of this disease on commercial sites in the UK is increasing, and a number of commercial varieties appear highly susceptible. As the disease has been easily controlled by varietal resistance in the past, it is possible that first symptoms are not recognised quickly, or that actions taken to control early infections are not swift enough to deliver effective results.

Objectives

This project aimed to document disease management practices currently used against tomato leaf mould (*Passalora fulva*) on UK nurseries, determine occurrence of fungicide resistant strains and, based on pathogen biology, and PE 018, propose changes to improve control. The specific objectives were:

1. Survey and visit nurseries to establish the prevalence of tomato leaf mould infection in the UK, and document treatments and practices currently used to manage the disease;
2. To collect isolates of *P. fulva* from affected crops and determine their sensitivity to current standard fungicides and potentially useful new products;
3. To establish a best practice guide for control of leaf mould in crops based on previous research, grower experience and the results of investigations and tests conducted in this project.

Summary

Objective 1: Current prevalence and management practices

Tomato leaf mould was reported on five of the six nurseries surveyed in 2016, with occurrences on sites in Cambridgeshire, Lancashire, West Sussex and Yorkshire. At least one site which was not monitored during this project also developed a tomato leaf mould infection A total of 16 different varieties were affected at these sites (Table 1), of which ten varieties made no claim of resistance to *P. fulva* and infection on these was not unexpected. Five varieties (Amoroso, Avalantino, Kierano, Piccolo and Sweetelle) that each claim resistance to race groups A to E were affected to some extent. This result provides strong evidence that races of *P. fulva* able to overcome resistance genes *Cf*-1 to *Cf*-5 are present in the UK. There was no information available on the resistance status of the remaining variety, Jester.

Table 1. Tomato varieties reported in grower questionnaires as affected by tomato leaf mould in 2016; several currently claim resistance to *P. fulva*

Variety	Breeder	Listed resistance to *P. fulva*
Amoroso*	Rijk Zwaan	A-E
Angelle	Syngenta	No resistance claimed
Avalantino*	Enza Zaden	A-E
Bamano	Syngenta	No resistance claimed
Campari	Enza Zaden	No resistance claimed
Garincha	Enza Zaden	No resistance claimed
Juanita	Monsanto/De Ruiter	No resistance claimed
Jester	Tozer	Unknown
Kierano*	De ruiter	A-E
Lipso	Clause	No resistance claimed
Papeletto	Rijk Zwaan	No resistance claimed
Piccolo*	Gautier	A-E
Solarino	Rijk Zwaan	No resistance claimed
Sunstream	Enza Zaden	No resistance claimed
Sweetelle*	Syngenta	A-E
Ternetto	Rijk Zwaan	No resistance claimed

*Varieties which claim resistance to *P. fulva* but were reported infected during 2016

The first outbreak of tomato leaf mould was reported to have occurred in May; the final outbreak did not occur until September. Leaf mould had been seen the previous year on five of the six sites. On three nurseries, the level of leaf mould was assessed between three weeks and three months after first symptoms had appeared. At this first assessment, the incidence of affected plants was over 50% on four of seven varieties assessed, with a mean severity of over 50 leaf spots/plant on two varieties. At a final assessment, near to the end of cropping, almost 100% of plants were affected on three varieties. On nursery A, the same variety Ternetto was grown in glasshouses of different ages, leaf mould severity was greatest in the new glasshouse (3 years old) and less in older houses (40 years old). No comparisons of crops in glasshouses of different ages were possible at sites B and C as different varieties were used in each house.

Plant protection products used against leaf mould were Amistar, Signum, Switch, Teldor and Serenade ASO (*Bacillus subtilis* strain QST 713), with Amistar considered the standard product. The total number of sprays applied ranged from zero in organic crops to upwards of ten. Spray volumes ranged from 1250-2500 L/ha. All respondents angled nozzles in order to treat the underside of leaves, the leaf surface with most stomata, through which *P. fulva* infects. In addition to plant protection products, measures used against leaf mould included: maintaining relative humidities below 85%; use of impermeable plastic sheeting rather than Mypex-type matting to cover the floor; removal of fixed plastic energy screens; de-leafing hard at the first sign of infection (combined with a fungicide spray, usually Amistar); removal of affected leaves from the house to a covered skip; use of varieties with claimed resistance to leaf mould; rotation of susceptible and resistant varieties to different locations in a house each year; use of Serenade ASO to extend spray intervals between conventional fungicides; end of season clean-up and disinfection of glasshouse and equipment.

Objective 2: Sensitivity to Plant Protection Products

Seven isolates of *P. fulva* were collected from UK crops and maintained in culture. Several of the isolates were found to carry the naturally occurring hyperparasite *Hansfordia pulvinata*. Attempts to clean these isolates of the hyperparasite were partially successful; any cultures with obvious *H pulvinata* were not used in tests. The isolates were tested for their sensitivity to Amistar, Switch, Prolectus, Reflect (isopyrazam), Mycostop (*Streptomyces griseoviridis)*, Prestop (*Gliocladium catenulatum)* and Stopit (calcium chloride) at label rates in a detached tomato leaf bioassay using benzamidazole agar to delay leaf senescence. Conventional fungicides were applied 3 hours and biofungicides 5 days and 3 hours before inoculation with *P. fulva* spores.

Disease development was poor with most treatments showing <2% leaf area affected at three weeks after inoculation when the experiment was terminated. Even the untreated control leaves had little disease. There was no significant differences between treatments. In a second experiment using a detached leaf bioassay, the fungicides Amistar, Comet (pyraclastrobin), Filan (boscalid) and Switch were each tested at four concentrations (0.1, 1, 10, and 100 ppm a.i.). At three weeks after inoculation, there was <1% leaf area affected on most leaves, including the untreated control, and there was no significant differences between treatments. Amistar used at the highest rate (100 ppm a.i.) was the only treatment on which no leaf mould developed. No firm conclusions can be drawn from these experiments. It is suggested that in any future work, experiments on product efficacy are carried out on whole plants and/or agar plate inhibition assays rather than a detached leaf assay; or that an improved leaf bioassay is sought which results in higher disease levels, to better allow discrimination between treatments.

Objective 3: Best practice guide

A best practice guide for control of leaf mould has been devised.

Key aspects are:

- Preventative spray(s) with suitable plant protection product(s) on varieties with no claimed resistance, and in houses with a history of the disease. If no preventative spray is used, very rapid action is required when the disease is first seen due to its epidemic potential.
- Reducing disease risk by careful monitoring of glasshouse relative humidity (RH) and avoiding prolonged periods above 85% RH; especially around the lower leaves where the disease usually starts. Regular de-leafing (weekly when appropriate) can aid air movement around lower leaves.
- Angle spray nozzles to treat the underside of leaves; check to ensure good spray coverage is achieved.
- Amistar can give good control but a decline in efficacy has been noted by growers with repeated use, likely indicating resistance developing. There is some evidence that Switch may also lose efficacy with time. Serenade ASO is useful, as a preventative, to stretch the interval between sprays of a conventional fungicide and thereby allow construction of a season-long programme, in situations where the disease starts early.
- Grower experience indicates there can be a benefit from using an impermeable plastic sheet to cover the soil rather than the Mypex-type matting; and to rotate the location of susceptible and resistant varieties in a house between years (do not grow a variety with no claimed resistance in an area where leaf mould occurred the previous year). One grower also removed all fixed plastic energy screens which they believed helped prevent the disease occurring at their site.
- Maintaining a high standard of hygiene: de-leaf hard and promptly when the disease occurs; remove affected leaves and debris to a covered skip outside the glasshouse; undertake a thorough end of season clean-up and treat the glasshouse structure and equipment with a suitable disinfectant.

Financial Benefits

- Knowledge of the frequency of tomato leaf mould strains resistant to Amistar (azoxystrobin) or other fungicides will inform design of plant protection programmes for improved control and prevent wasteful use of products likely to be ineffective.
- Severe infections due to high disease incidence have resulted in crops being removed from glasshouses before end of cropping. Identification of likely gaps/weaknesses in current leaf mould control strategies will enable growers to implement changes with a good chance of improving control.
- Reduction in risk to human health from leaf mould epidemic spore-loads, reducing associated sick leave costs and staff absences.

Action Points

- Prevention of infection by managing the glasshouse environment is easier than eradicating the disease – epidemics can occur quickly given the chance.
- Rectify any leaks or areas in the glasshouse where water pools; this can increase humidity in the area and increase the chance of a hotspot occurring for *P. fulva.*
- Using impermeable plastic to cover soil in glasshouses is likely to prevent moisture escaping the soil, and maintain a cleaner glasshouse environment than permeable Mypex-type matting, providing there is no pooled water due to leaking irrigation/vents.
- Effective clean-up at crop turnaround will lower the inoculum present on site – a thorough clean-up can mean problems begin later in the season, if at all.
- If infection is considered likely, for example if a nursery was infected and/or a known highly susceptible variety is being grown the previous year, aggressive humidity control will reduce the chance of re-infection and spread – monitor relative humidity (RH) around the lower leaves to provide the most relevant information on leaf mould risk.
- Good resistance management is especially important in controlling *P. fulva* – potential resistance to Amistar was already reported by some growers, and in the 2016 season Switch was reported to be less effective than previously
- Use of biological fungicides (e.g. Serenade ASO) to space out conventional sprays can extend a spray programme
- If a variety with no claimed resistance to leaf mould is grown, do not site this variety in areas where leaf mould was observed the previous year.

SCIENCE SECTION

Introduction

Tomato leaf mould caused by *Passalora fulva* (previously *Cladosporium fulvum*) is a destructive foliar disease of increasing importance in the UK. Outbreaks have occurred most years over the last decade and affected a range of varieties. Previously well controlled by genetic resistance, the new outbreaks appear to be caused by the cultivation of varieties with no claimed resistance and the emergence of strains capable of overcoming the resistance genes deployed in current varieties. Currently there is no easy method to identify strains; the classical approach is to determine pathogenicity of isolates by inoculation on to a differential set of tomato varieties, testing one isolate at a time, which is time-consuming and costly; no molecular methods have been developed to our knowledge.

New strains of the pathogen have recently been identified in Japan (Kubota *et al.,* 2015; Iiada *et al.,* 2015), China (Li *et al.,* 2015) and Korea (Lee *et al.,* 2013) for which no varietal resistance is in place. The disease is also causing considerable problems in Argentina (Rollan *et al.,* 2013). Identifying the race of the pathogen is an expensive process, involving the growing of numerous tomato varieties that possess different resistance genes (Li et al., 2015). Identification of races 1 and 3 is also not perfect with this system (Kishi, 1962). As new *Cf* genes conferring host resistance are introduced into commercial tomato varieties, new strains of *P. fulva* evolve. New strains present in Japan are distinct from those developing in other parts of the world, but follow a similar pattern in response to resistant tomato varieties (Iida *et al.,* 2015). The rapid adaptation of *P. fulva* presents a considerable problem for tomato breeders, and in a recent screen of 41 tomato genetic resources tomato leaf mould was found to infect all 41 to some degree, though some did show a high level of tolerance (Su *et al*., 2014).

P. fulva is a hemibiotrophic pathogen, starting the infection process by entering plant stomata and feeding as a biotroph. However, the pathogen is unable to penetrate host cells to gain nutrition and survives in the apoplast. Infected tissues show fungal proliferation against the apoplastic sucrose gradient (Thomma *et al.,* 2005). It is theorised that it is depletion of carbon and/or nitrogen sources that induce starvation-regulated genes and cause the pathogen to switch to a necrotrophic lifestyle (Coleman *et al.,* 1997).

Additionally, although Amistar (azoxystrobin) has given good control in some crops, grower reports indicate fungicide resistant strains can develop within a few years. Previous work, PE 018, showed a number of other products including Switch (cyprodinil + fludioxonil) and Signum (boscalid + pyraclastrobin) also give good control when used preventatively. The new product Prolectus (fenpyrazamine) warrants evaluation as the closely related fungicide Teldor (fenhexamid) gave some control in PE 018. Similarly, one or more Succinate dehydrogenase inhibitor (SDHI) fungicides submitted for authorisation to use on protected tomato warrant laboratory evaluation as these are generally fungicides with broad spectrum activity.

The disease also affects organic crops, where use of Amistar and other conventional fungicides are not permitted by the Soil Association. Nursery sanitation and hygiene measures between crops, choice of variety and glasshouse environment control are critical in this situation. Spores of *P. fulva* appear to be very resistant to dryness and low temperatures, and are believed to survive in a dormant state from one crop to the next. The fungus can also survive saprophytically in dried leaf debris (Yan *et al*., 2008). A number of disinfectants were shown to be effective against *P. fulva* in PE 018, however effective crop clean up remains problematic on some commercial sites, with re-infection commonly occurring year on year.

Possibly the results obtained in PE 018, and appreciation of the unusual biology of *P. fulva*, have not been exploited by growers to best advantage for maximal effect as the disease has continued to be a problem in the UK. Further effort to identify gaps/weaknesses in current control practices and establish improved targeted, integrated control measures is required, as incidence of this disease on commercial sites in the UK is increasing, and a number of commercial varieties appear highly susceptible. As the disease has been easily controlled by varietal resistance in the past, it is possible that first symptoms are not recognised quickly, or that actions taken to control early infections are not swift enough to deliver effective results.

As well as the active ingredients included in PE 018, straight sprays of both boscalid (included in PE 018 in Signum with pyraclastrobin) and pyrimethanil have been found to offer control (Chen *et al*., 2013). In recent research, a number of active ingredients not yet commercially available have been shown to have some efficacy against *P. fulva*. The potential of two *Streptomyces* species as biocontrols against tomato leaf mould has been investigated successfully, with *Streptomyces albidoflavus* (Chen *et al.,* 2015) and *S. lavendulae* (Gao *et al.,* 2016) both showing promise. Though these antifungal agents were both novel strains, a strain of *Streptomyces, Streptomyces griseoviridis* strain *K*61, is already permitted for use in tomato as Mycostop. The potential of the commercial product Mycostop as a biopesticide against *P. fulva* warrants further investigation. Inhibitory effects of tomatine on tomato leaf mould have also been recently investigated showing a possible role in varietal resistance (Dow & Callow, 1978), and can be purchased as a laboratory reagent but is not available as a plant protection products (PPP). Additionally, liquid waste produced by steam-exploded detoxification of *Miscanhtus sinensis,* a byproduct of the biofuel industry, has been shown to supress *P. fulva* when applied preventatively (de Corato *et al.,* 2014), and a bacterial strain known as AYM-18 has been shown to be antagonistic (Zhang *et al.,* 2014).

It is thought that a detached leaf assay may be more suitable for *P. fulva* than an agar plate assay due to its hemibiotrophic lifestyle. For example, it has been observed that the fungus does not produce disease effectors *in vitro*, or does so at a reduced rate (de Wit *et al.,* 2009 in Vleeshouwers & Oliver, 2013). In 2013, a *Cladosporium* leaf spot (related to *P. fulva*, previously known as *C. fulvum*) was reported from tomato glasshouses in China (Huang *et al.,* 2013). Thus knowledge of the efficacy of currently available and pipeline plant protection products against this group of pathogens may become more valuable in future.

Materials and methods

Objective 1 - Survey and visit nurseries to establish the prevalence of tomato leaf mould infection in the UK, and document treatments and practices currently used to manage the disease

In 2016, three commercial production sites were visited and leaf mould incidence and severity was assessed. These sites were selected because they had commonly experienced tomato leaf mould in previous seasons. Additionally, growers were questioned about the past and present experiences with the disease. When infection occurred on site the actions taken to control it and their perceived efficacy were noted.

Disease assessments

Site visits were planned to occur once before initial infection, once after leaf mould had occurred, and once towards the end of the season. On arrival at each site, it was established if leaf mould infection was present on site, and if so where the outbreak was located. Any pesticides sprayed to the affected areas were recorded, as well as other disease management activities undertaken by the grower.

For each crop area affected by leaf mould, an individual assessment of 100 plants chosen at random was carried out. Plants were assessed for leaf mould incidence, and severity of the disease. Severity was recorded as the number of individual leaf mould lesions present (Figure 2). This was recorded for the whole plant, but also for the lowest two leaflets individually as this is where the most severe infection tends to remain. In addition to this, the height infection had reached on plants was recorded, in terms of the number of leaves from the bottom. At the final visit of the year, when plants had all had their heads removed and only a few leaves remained, it was noted if the entire plant was affected. If lesions were very young, and sporulation had not yet begun, this was also noted.

Figure 2. An example of the underside of a badly infected leaf sampled from a commercial crop in 2016

Environmental data (hourly temperatures and humidities) was also sought from growers, in order to link any outbreaks with higher than usual humidities.

Notes on control

As disease progress and response to treatment was monitored on commercial sites, the perceived efficacy of control options employed was monitored. The situations where control was relatively successful versus where disease control proved difficult were compared.

Leaf mould survey

As well as recording practice at the sites visited, other commercial growers were also approached for their comments, experiences and opinions with tomato leaf mould, including those who had never experienced an outbreak. In total, six commercial growers responded to requests for this information, including the three sites monitored as part of this investigation. Questions on the impacts of the disease, disease distribution, glasshouse age, spray application equipment, and products used were included. A copy of the survey questions supplied to the six participating growers is included in Appendix 1.

Objective 2 - To collect isolates of P. fulva from affected crops and determine their sensitivity to current standard fungicides and potentially useful new products

Isolate collection

Over the 2016 season, a number of isolates were collected from commercial nurseries. Isolates were taken from several different scion varieties which also differed in terms of the fungicide treatments they had been treated with. *P. fulva* was isolated in each case by picking off spores from active leaf lesions and streaking onto agar plates (potato dextrose agar, amended with the antibiotic streptomycin (0.25 g/100ml water), using a sterile needle.

Trial set-up

Two trials were established in the pathology laboratory at ADAS Boxworth to examine: 1) the efficacy of selected fungicides and biofungicides when applied at their recommended commercial rates; and 2) the possibility of resistance to selected conventional fungicides currently in use.

Fungicides were tested on detached tomato leaves of a susceptible variety, cv. Bamano. Upper leaves that were free of infection were sourced from a commercial site, and each plot was made up of single leaves held in a clear plastic box. Leaves were placed with their undersides facing upwards (as this is where the fungi infects), and a piece of cotton wool soaked in sterile distilled water (SDW) was placed in each sealed box to maintain adequate humidity for infection. Leaves were placed into sterilin tubes containing tap water agar (1L water + 15g agar), amended with benzamidazole agar at 30 mg/L (Limpert *et al.,* 1988) to slow senescence and maintain their green colour throughout the trial. An example of the experimental set up can be seen in Figure 3.

Figure 3. Leaves were placed upturned in plastic boxes, tap water agar amended with benzamidazole was used to slow senescence – ADAS Boxworth, 2016

Fungicide products were applied preventatively, prior to inoculation, using a calibrated hand sprayer. Inoculum was also applied using a hand sprayer, having been bulked up on agar plates (PDA+S) with a 4ml spore suspension ($1x10^5$ spores/ml) applied to the whole leaflet. Plates with actively growing *P. fulva* were placed under UV lamps for a week prior to inoculation to encourage sporulation. Plates were flooded with SDW, and spores agitated into suspension. This spore suspension was then adjusted to 1×10^5 spores/ml in concentration using a haemocytometer. It should be noted that isolates of *P. fulva* commonly carry a naturally occurring hyperparasite (*Hansfordia pulvinata*) (Figure 4). Care was taken to avoid plates with abundant *H. pulvinata* present, but it was undoubtedly present in some isolates. Each of the two experiments below was replicated six times.

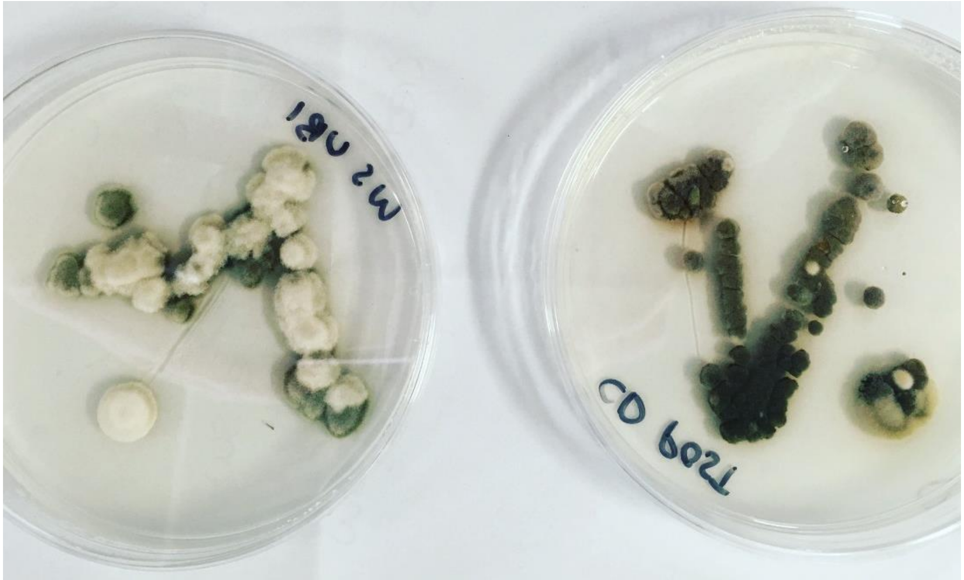

Figure 4. Isolates of *P. fulva* (olivaceous green) growing on PDA+S plates show the presence of *H. pulvinata* (white); the left hand plate is badly affected, whereas the right hand plate has only a small amount of the hyperparasite present.

Efficacy trial

The treatments applied included Amistar and Switch, as these could be considered current industry standards for control of *P. fulva*. In addition to these treatments, a number of products that have been approved on tomato since the last research project on *P. fulva* concluded (PE 018) or that could be promising based on recent academic literature were tested. Treatments and the rates at which they were applied are listed in Table 2. Conventional products were applied once, on the morning before leaves were inoculated in the afternoon. Biological products were applied both five days before inoculation, on 7th October, and again the day before, the 12th October. Inoculation took place on 13th October, 2016.

Table 2. Rates and treatments, including biologicals used to determine efficacy against *P. fulva*

Trt	Product	a.i.	Commercial rate
1	Untreated	Untreated	-
2	Amistar	Azoxystrobin	0.1 L in 100 L water
3	Switch	Cyprodinil + Fludioxonil	1 kg/ha
4	Prolectus	Fenpyrazamine	120 g in 100 L water
5	Reflect	Isopyrazam	0.1 L in 100 L water
6	Mycostop	*Streptomyces griseoviridis*	32 g in 40 L water
7	Prestop	*Gliocladium catenulatum*	0.5 kg in 100 L water
8	Stopit	Calcium chloride	5 L/ha

Resistance testing

Products currently in use for leaf mould control were applied at a range of concentrations (0-100 ppm). Treatments are listed in Table 3. Fungicide solutions of the appropriate strength were calculated based on the weight or volume active ingredient per litre, listed on the label of formulated products. As resistance to Amistar has been reported, Signum was included split into its constituent active ingredients to determine if a more general resistance to strobilurins was present, and if the relatively new functional group of SDHIs were effective. Products were applied once, on 12th October, before plots were inoculated on the 13th.

Table 3. Concentrations of treatment actives used to examine *P. fulva* isolates for resistance

Trt	**Product**	**a.i.**	**Rate**	**Concentration (ppm)**
1	Untreated	Untreated	-	0
2	Untreated	Untreated	-	0
3	Amistar	Azoxystrobin	1 ml/L	100
4		250 g a.i./L	0.1 ml/L	10
5			0.01 ml/L	1
6			0.001 ml/L	0.1
7	In Signum (Comet)	Pyraclastrobin 200 g a.i./L	1 ml/L	100
8			0.1 ml/L	10
9			0.01 ml/L	1
10			0.001 ml/L	0.1
11	In Signum (Filan)	Boscalid 500 g a.i./L	1 ml/L	100
12			0.1 ml/L	10
13			0.01 ml/L	1
14			0.001 ml/L	0.1
15	Switch*	Cyprodinil+Fludioxonil	1 ml/L	100
16		375 g a.i. /L	0.1 ml/L	10
17			0.01 ml/L	1
18			0.001 ml/L	0.1

*ppm value was calculated based on the amount of cyprodinil in the formulation

Assessments

Following inoculation, leaves were checked daily for first symptoms of *P. fulva*. Once these were seen, both experiments were assessed a total of four times. Both incidence of *P. fulva* and severity of *P. fulva* (% leaf area affected) were assessed.

Objective 3 - To establish a best practice guide for control of leaf mould in crops based on previous research, grower experience and the results of investigations and tests conducted in this project

The findings of work under Objectives 1 and 2 were collated, and a grower focussed best practice guide has been produced. This can be accessed via the AHDB website, and its text is included in Appendix 5.

Results and discussion

Objective 1 - Survey and visit nurseries to establish the prevalence of tomato leaf mould infection in the UK, and document treatments and practices currently used to manage the disease

Table 4 summarises the disease assessments carried out on commercial sites in 2016, where leaf mould was reported by three commercial growers. Each site was assessed twice, soon after the disease was first observed, and again towards the end of cropping. At site A, infection levels at the first visit, 3 weeks after the disease was first noticed, ranged from 6% to 94% of plants affected, with up to 56 leaf spots per plant. Disease incidence, severity and sporulation were all notably lower in the middle-aged glass (15 years old), than in the newest glass (3 years old). Application of a Natugro programme, a method of growing which exploits microorganisms around plant roots benefiting the plant, appeared to have no effect on disease incidence and appeared to increase disease severity. At the second assessment 6 months later, there was a high disease incidence (>59% of plants) in all houses. Disease severity was notably much greater in the new glasshouse than in the two middle aged and one old house.

At site B, no conclusions could be drawn as to the effect of glasshouse age as different varieties were grown in the two houses. At the first assessment on 1st September, leaf mould incidence and severity was similar on cv. Piccolo in middle aged glass and cv. Sophie Jane in newer glass, with 10-16% of plants infected, at a low disease severity. Two months later leaf mould incidence and severity were much greater on cv. Piccolo in the older glasshouse than on cv. Sophie Jane in the newer glasshouse.

At site C, a high incidence (97-100% of plants) were found to be affected in both cvs Piccolo and Amoroso on 27th September, 3 months after the disease was first noticed. Disease severity was greater on Amoroso than Piccolo. Two months later, disease severity on cv. Amoroso had declined due to removal of lower, badly affected leaves.

All varieties at site C and several from sites A and B developed leaf mould to some extent during 2016 including several susceptible varieties (e.g. Ternetto) and those which claim resistance (e.g. Amoroso). This indicates the extent of susceptible cultivars is larger than we currently know and that varieties claiming resistance may only be partially resistant or no longer resistant at all. In addition, infection was found in glasshouses of a variety of ages, and the most severe infection was observed under the newest glass. Though older glasshouses are more likely to have leaks and standing water, newer glasshouses form a more sealed environment, and the addition of screens also serves to increase humidity. This can create an environment extremely favourable for *P. fulva* development. It should be noted that the later assessment dates at each site had fewer leaves remaining on the plants than at the earlier assessments, and as such numbers recorded are generally lower.

Table 4. Occurrence of leaf mould across the season from three commercial sites comparing ages of glasshouse with disease incidence and severity

Location	Variety	Date assessed	% plants affected	Av. no. lesions per plant	Av. no. lesions on lowest 2 leaves	Av. Height (no of leaves from base) reached on plant*	% Sporulating	% plants where all leaves affected**
Site A								
Newest glass (Natugro)	Ternetto	25-May	86	7.9	3.9	3.9	86	-
Newest glass (not Natugro)	Ternetto	25-May	94	56.2	25.4	5.7	94	-
Middle aged glass, end 4 rows	Ternetto	25-May	6	0.1	0.1	1.2	0	-
Newest glass	Ternetto	28-Oct	100	343.9	76	5.9	72	92
Middle aged glass, far house	Ternetto	28-Oct	62	4.4	3	2.3	59	8
Middle aged glass, end 4 rows	Ternetto	28-Oct	59	3.9	2.9	2.6	54	10
Old, low glass	Ternetto	28-Oct	70	8.9	3.6	3.3	70	1
Site B								
Middle aged glass	Piccolo	01-Sep	10	0.9	0.5	2.2	6	-
Newer glass	Sophie Jane	01-Sep	16	0.6	0.4	1.9	13	-
Middle aged glass	Piccolo	02-Nov	86	17.8	13.9	3.2	85	30
Newer glass	Sophie Jane	02-Nov	2	0.02	0.02	1	2	0
Site C								
Middle aged glass	Piccolo	27-Sep	97	44.8	24.4	4.4	98	-
Middle aged glass	Amoroso	27-Sep	100	100	100	8.8	100	-
Middle aged glass	Amoroso	07-Dec	97	59.9	43.9	2.9	97	97

*of those affected

**only assessed at the end of the season, as plant heads had been removed and only approx. 5 leaves remained

Several growers were approached and asked to fill out a questionnaire on their treatment against leaf mould. Six responses were returned, including from the original three growers visited. The responses and recommended control practices are summarised in Table 5.

The six growers reported that historically the original occurrence of tomato leaf mould on their sites happened between 2009 and 2014 and the disease has continued to be a problem for most since this time. The first occurrence of the disease during 2016 differed between sites with site A becoming infected earlier (May) than the others (June-September). Two of the six sites controlled the disease very well, with one site only minimally affected whilst the other site, growing both organic and conventional crops, was not infected during 2016 at all. Other sites suffered varying levels of the disease which persisted throughout the entirety of the season.

During 2015 one grower underestimated the potential damage tomato leaf mould could cause and was forced to withdraw a crop three weeks early, at considerable cost. All sites questioned de-leafed the crop, with some doing so preventatively, and others doing so once symptoms occurred. Typically growers who do not spray preventatively do so once the plants become symptomatic. Two growers preferred to wait before treating and controlled the disease with aggressive humidity control (maintaining a HD below 3.5 g/m^3) and hard de-leafing, conserving sprays for later in the season.

Amistar was sprayed by all conventional growers and alongside Teldor was generally sprayed before Switch, which is often saved for later use due to its limitation of two applications per year. One grower reported Amistar was not as effective as in the past as it used to eradicate the disease, however during 2016 the disease was merely held back. This site and one other which also experienced widespread infections in 2016 used biological fungicides such as Serenade ASO to successfully keep the disease in check. One site reported poor results from biological fungicide use in the past and a reluctance to apply more as this would increase humidity within the crop. The other sites questioned did not report any issues with Amistar.
During 2016, site C suffered infection on all varieties, including those with claimed resistance. This was found to some degree at other sites. This indicates the potential presence of unknown isolates able to infect currently resistant varieties. However, conversely other growers completely agree with the variety resistance claims and have not seen infections on resistant varieties, and two growers have recently switched to growing mainly or only resistant varieties. Sites which rotate the location of resistant and susceptible varieties each year have reported effective control.

At the end of the season, the three growers were asked to report on the whole season. Those which managed the disease effectively will continue with their existing crop management strategies into 2017. One site which experienced issues has now switched to growing only the resistant variety Funtelle. Other changes by growers include better aggressive humidity control, de-leafing, improved ventilation and earlier treatment.

It should be noted that the knock-on effects of good disease control are not simply increased yields. Lower inoculum levels makes the challenge of total eradication of viable spores much more achievable at clean-up benefiting the following crop. Less severe disease can lead to the delaying of spray application or can even reduce the number of sprays required that season and lower spore numbers will reduce risks to human health.

Table 5. Different control methods and feedback in managing tomato leaf mould provided by responses to the growers' questionnaire

Control measure	**Results and growers feedback**
Timing of PPP application	Growers who treated preventatively or as soon as symptoms developed will have better control over the season than those who treated once symptoms had developed further.
Humidity	Three growers tried to keep relative humidity below 85% with one reporting that although expensive the level of disease control is well worth the cost. One that used the Dutch 'new way of growing' maintaining a high even humidity suffered a uniform infection across their glasshouse. Several growers use humidity deficit rather than relative humidities to control their glass house humidity, but should routinely check RH levels, especially around the lower leaves.
Disease 'hot-spots', including leaks and areas of pooled water	Disease hot-spots occurred earlier in the year and *P. fulva* will spread from these to the rest of the glasshouse unless addressed as soon as possible. Growers who didn't address these suffered epidemics due to inoculum from these original foci. A grower reported that the use of impermeable plastic sheeting rather than Mypex-type matting lowered disease levels by reducing humid microclimates and preventing spore entry from the soil. Reducing pooled water within houses was also claimed to reduce disease levels.
Cultural control and de-leafing	All growers de-leafed to control leaf mould, many de-leafing hard when the disease was discovered, combining this with a fungicide spray, usually Amistar. Several noted that de-leafing higher up the plant than usual has a more positive effect, increasing airflow whilst removing *p. fulva* inoculum. Two sites used aggressive humidity control, alongside de-leafing, maintaining a humidity deficit below 3.5 g/m^3 to extend the time before the first treatment spray. All debris should be removed and be covered away from the glasshouses.

Varietal resistance, including crop rotation	Generally speaking growers had more success with varieties that claimed *Cf* resistance however several of these varieties did develop leaf mould symptoms to some extent despite currently claiming resistance (Table 6). Some growers stopped growing varieties with which they had had problems with leaf mould in the past, focussing now on resistant varieties such as Funtelle. Rotation of susceptible and non-susceptible varieties to different locations at sites each year has been claimed to provide significant disease control. Planting susceptible varieties away from previous sites of high disease should reduce the risk of disease occurrence and severity.
Chemical control	Amistar was used by all conventional growers and is considered the industry standard. Growers reported varying levels of control, some stating that use of Amistar simply delayed or knocked-back the disease rather than eradicating it, as it had done in the past. Use of Amistar twice in a row, followed by controlling the disease with other actives such as Signum and Teldor later provided good results. Switch was often saved until the end of the season due to its limitation of two applications. The efficacy of Signum against *P.fulva* has been noted to be declining by some growers. Other chemicals included copper and sulphur were used and had no noticeable effect on the disease.
Biological control	Relatively few growers had used biological controls to control leaf mould. Many of those that had reported little or no effect although they may have had some effect on Botrytis. Biologicals can be successfully used to extend spray intervals with one grower stating that they would have experienced significant issues without the use of Serenade ASO to do so. Another grower reported using biologicals curatively with no success however they are most effective when used preventatively.
Treatment sprays and equipment	The six growers used a variety of different spray practices to control leaf mould. Spray equipment varied from the manual ripa sprayer, through the semiautomatic, to fully automated robotic systems, utilising water volumes between 1250 and 2500L/ha. Nozzle types also differed including flat fan and hollow cone spray tips. Many only targeted the upper leaves whilst some treated the entire plant. When possible treat the entire plant and ensure to target inoculum sources most commonly present on the lower leaves. All growers angled the spray nozzles to target the underside of the leaves where disease is most prevalent. Most treated to run-off, but not all. Those that had better coverage likely achieved better disease control.
End of season clean-up	A wide variety of methods and disinfectants were used at clean-up. The sites which have the most comprehensive clean up methods targeting all parts of the glasshouse architecture will likely have the most effective control. In all cases as much plant debris was removed as possible. One grower reported that the disease was re-introduced into a house by a piece of equipment that was not cleaned as it was stored elsewhere during the clean-up process. Generally glass from older houses were noted to be more likely to develop the disease than newer houses which might be in part due to increased difficulty in cleaning older leakier glass.

A grower's best practice-guide providing guidance on how to manage the disease using cultural and chemical measures, effective crop husbandry, resistant varieties and plant protection products has been developed.

Table 6. Tomato varieties reported in grower questionnaires as affected by tomato leaf mould in 2016; several currently claim resistance to *P. fulva*

Variety	Breeder	Listed resistance to *P. fulva*
Amoroso*	Rijk Zwaan	A-E
Angelle	Syngenta	No resistance claimed
Avalantino*	Enza Zaden	A-E
Bamano	Syngenta	No resistance claimed
Campari	Enza Zaden	No resistance claimed
Garincha	Enza Zaden	No resistance claimed
Juanita	Monsanto / De Ruiter	No resistance claimed
Jester	Tozer	Unknown
Kierano*	De ruiter	A-E
Lipso	Clause	No resistance claimed
Papeletto	Rijk Zwaan	No resistance claimed
Piccolo*	Gautier	A-E
Solarino	Rijk Zwaan	No resistance claimed
Sunstream	Enza Zaden	No resistance claimed
Sweetelle*	Syngenta	A-E
Ternetto	Rijk Zwan	No resistance claimed

*Varieties which claim resistance to *P. fulva* but were reported infected during 2016

Environmental monitoring

The optimal growing conditions for tomato leaf mould are between 22-24°C and relative humidities of 85% or above. The disease will develop more slowly between 12-22°C and above 24°C and so may exist on many sites, but not necessarily be a problem. When environmental conditions become more conducive to disease development outbreaks can occur rapidly as a level of inoculum already exists within the crop. Current practice for most growers is to control glasshouse humidity based on humidity deficit (HD). HD is expressed as the difference between the amount of moisture in the air and how much moisture the air can hold when fully saturated, and this changes with temperature. HD control can be used to optimise plant development and growth through promoting transpiration. Nowadays growers tend to use HD to control their glasshouse humidity, with some growers aggressively controlling HD levels to between 2.8 and 3.5 g/m^3. Growers which controlled in this way had little or no issue with the disease since 2014. Some growers also use RH as this provides a better indication of the risk of condensation forming in the crop which relates better to disease development.

Temperature and humidity data were provided by three sites visited. Site A had glasshouses of varying ages, 3, 15 and 40 years old, and tomato leaf mould was originally detected in the newest glasshouse on May 2nd before spreading to the other houses 3 weeks later. Environmental data for the entire season for all the glasshouses at Site A are located in Appendix 2 and show that throughout the majority of the season conditions were favourable for leaf mould, especially between June and September.

Disease symptoms will usually appear around one week after infection. Under optimal conditions the disease cycle will take 14 days to complete. Figures 5-9 show the humidity and temperature conditions two weeks prior to the first time leaf mould symptoms were detected in each house. Dotted lines on each graph denote the HD values provided by growers which were reported to give good disease control (2.8 and 3.5g/m^3). HD conditions below 2.8g/m^3 indicate territory where humidity conditions are much more conducive to disease development.

The earliest symptoms on Site A were detected on May 2nd 2016 and were seen in the three year old glasshouses 5 and 6. These houses were considerably more sealed than the others at Site A and conform to the 'New Dutch way of growing'. Temperatures were favourable but not optimal for disease development, 18.8°C for House 5 and 19.5°C for House 6, and similar to those seen in the other houses even though this outbreak occurred three weeks prior to being seen in houses 1-4. Both houses were much more humid than the others on site, with the HD in house 5 below 2.8g/m^3 for 78% of the time (Figure 5). House 6 was similar, but not as extreme, beneath 2.8 g/m^3 for 70% of the 14 days before symptoms appeared (Figure 6). The grower reported that leaf mould came in early and uniformly in these houses and affected 90% of the plants at the first visit and it is likely that the disease spread across the whole site from houses 5 and 6. The initial source of infection is unknown but could be due to several different factors; poor end of season clean-up; introduction of infected material or unsterilized equipment from the previously heavily infected crop; reuse of Mypex-matting from the previous year; transfer of spores from the staff on site and the presence of a crop (Ternetto) which is susceptible to the disease.

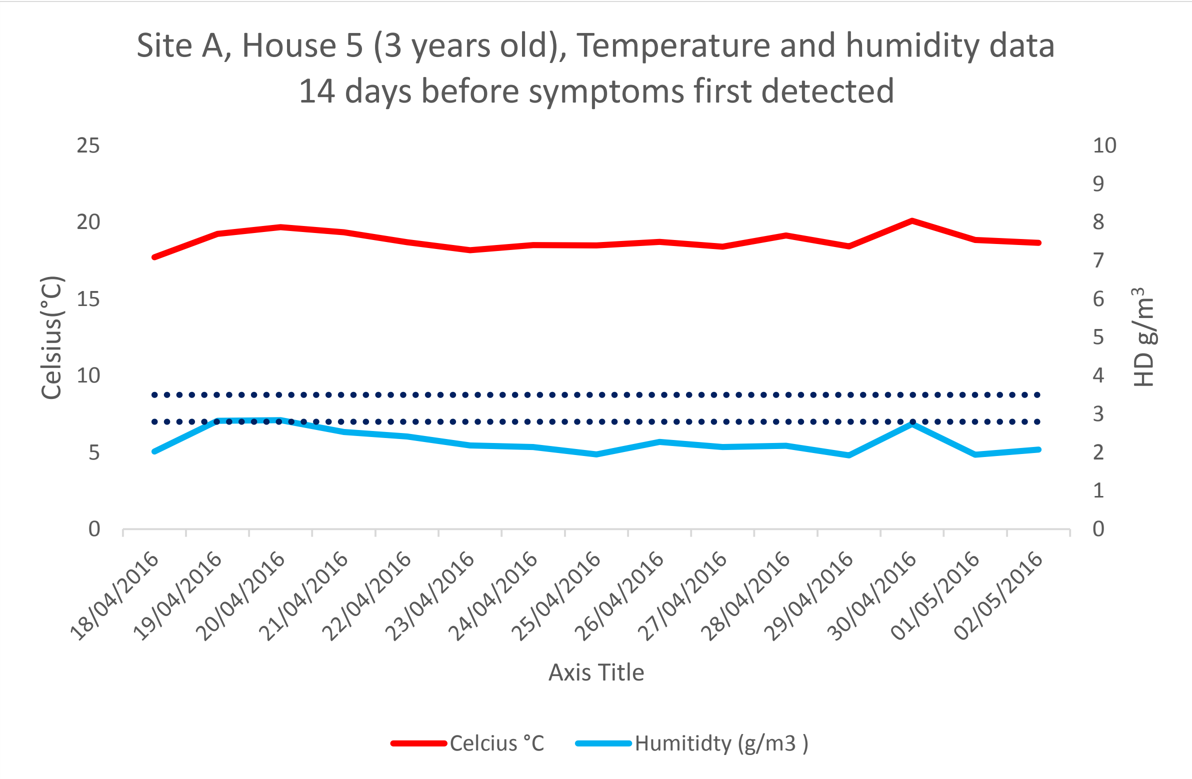

Figure 5. Temperature and humidity conditions two weeks before initial tomato leaf mould conditions were seen in House 5. Dotted lines on each graph denote the HD values provided by two growers which were reported to give good disease control (2.8 and 3.5g/m^3).

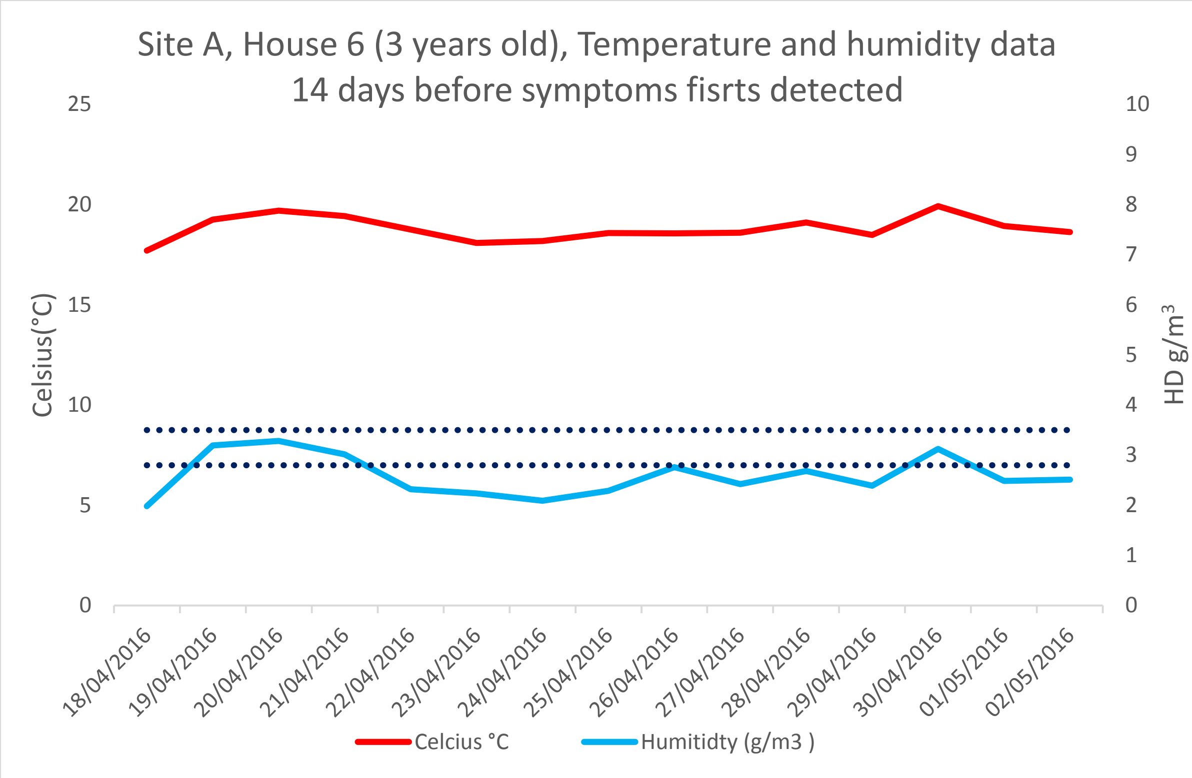

Figure 6. Temperature and humidity conditions two weeks before initial tomato leaf mould conditions were seen in House 6. Dotted lines on each graph denote the HD values provided by two growers which were reported to give good disease control (2.8 and 3.5g/m^3).

The disease was first seen in House 1 and 2 on May 25th 2016, three weeks after the initial outbreak of the disease at Site A. These houses were much older than Houses 5 and 6 (40 years old compared to 3 years old) and did not experience HD values below 2.8g/m^3 to the extent to which Houses 5 and 6 did (Figure 7). HD values below 2.8g/m^3 were recorded only 67% of the time in House 1 and 68% in house 2. The mean temperature over this time was slightly cooler than Houses 5 and 6 at 18.5°C. No temperature data was received for House 2.

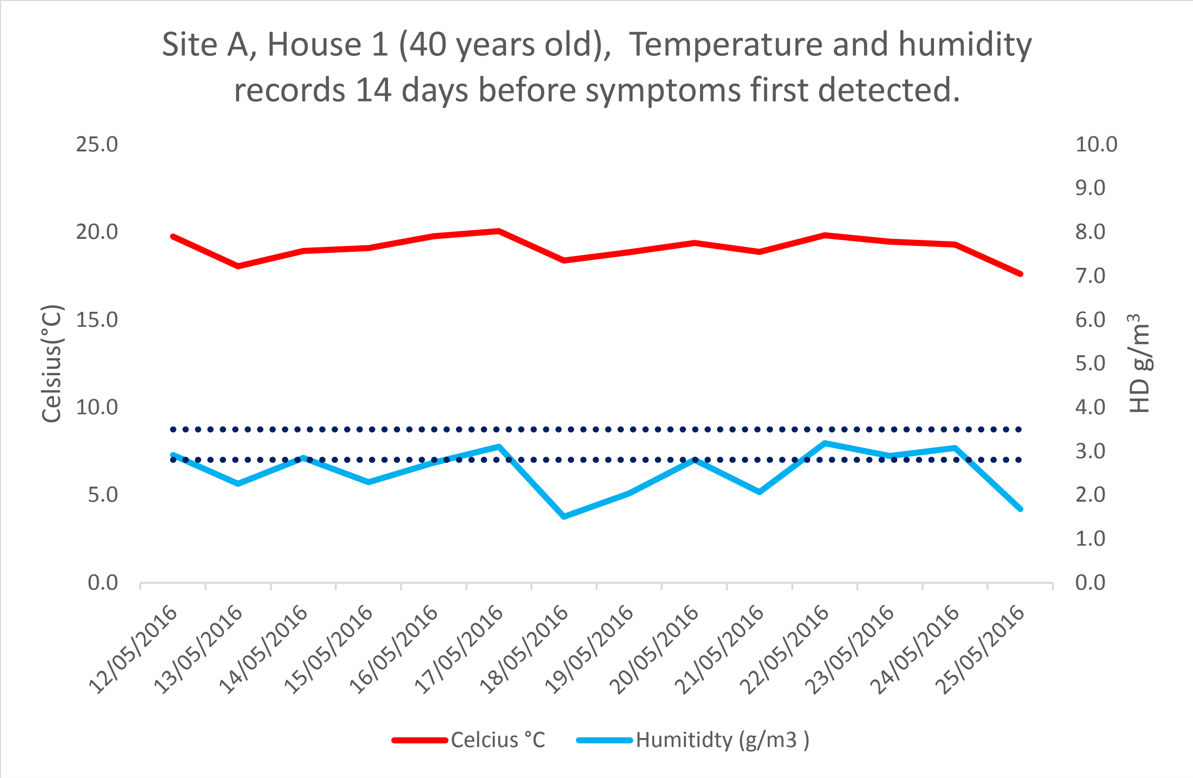

Figure 7. Temperature and humidity conditions two weeks before initial tomato leaf mould conditions were seen in House 1. Dotted lines on each graph denote the HD values provided by two growers which were reported to give good disease control (2.8 and 3.5g/m^3).

In houses 3 and 4 (15 years old) the disease was first seen around the 25th May 2016, the same time as seen in the older houses. The average temperatures recorded in these houses were also similar to those seen in the other houses, 18.8°C in both. Humidity values in House 3 reveal that a greater percentage of HD values lie in the range between 2.8 and 3.5g/m^3 than house 1, but a large proportion (64%) of the HD readings were still below 2.8g/m^3 (Figure 8). The HD values of House 4 were even greater than those seen in House 3 with HD values below 2.8 g/m^3 for 69% of the two week period (Figure 9). These differences between houses are likely due to the quality of the glasshouse architecture. Older glasshouses tend to be leakier and are more at risk of water pooling and disease hot-spots forming, but often have increased air flow which may reduce overall humidity.

Overall all houses on Site A, whilst not having optimal conditions for tomato leaf mould, did have conditions favourable to the disease.

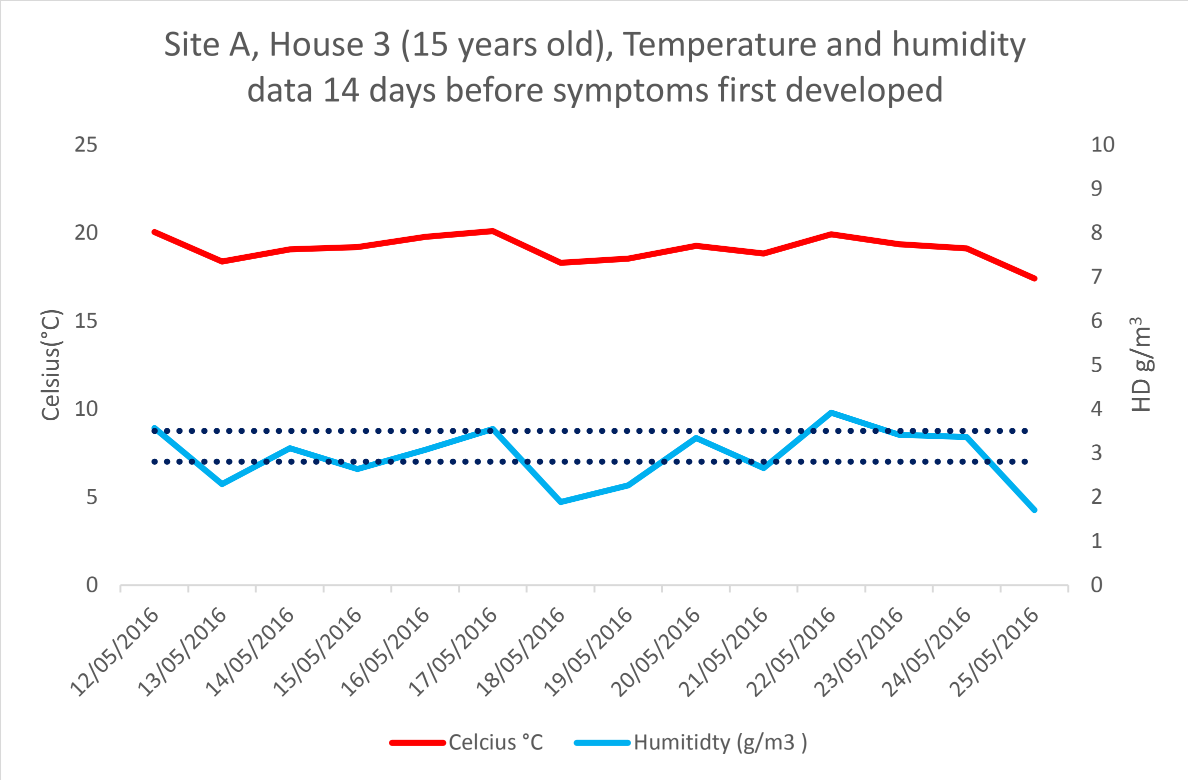

Figure 8. Temperature and humidity conditions two weeks before initial tomato leaf mould conditions were seen in House 3. Dotted lines on each graph denote the HD values provided by two growers which were reported to give good disease control (2.8 and 3.5g/m^3).

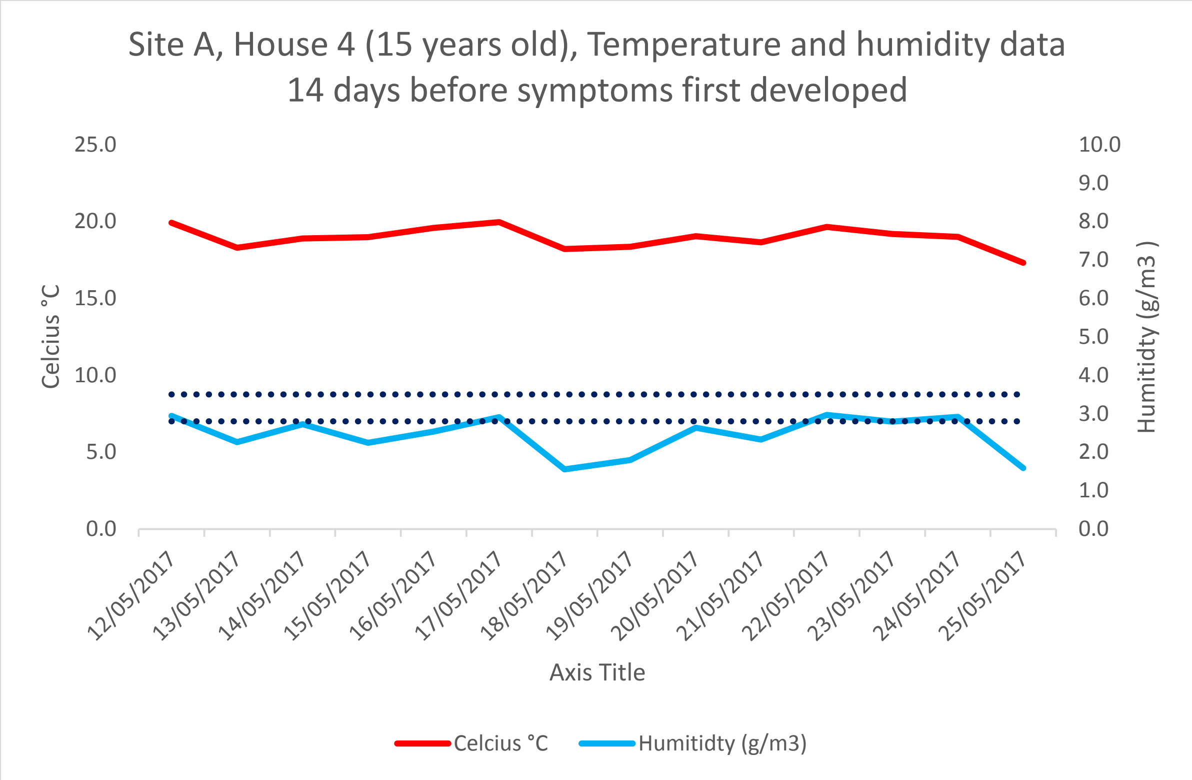

Figure 9. Temperature and humidity conditions two weeks before initial tomato leaf mould conditions were seen in House 4. Dotted lines on each graph denote the HD values provided by two growers which were reported to give good disease control (2.8 and 3.5g/m^3).

Site B supplied monthly averages for temperature and RH rather than HD values. Infection at this site was restricted to a few hotspots only, and not across the entirety of monitored areas (Figure 10). These smaller outbreaks appeared to occur in areas that had a combination of a susceptible (though not reported as such) variety and more damp and humid conditions, and where Mypex had allowed soil and moisture to pass through it into the glasshouse. Figure 10 shows that conditions for leaf mould development did occur on this site with mean RH values at or just above 85% (dotted line on Figure 10) over a period of 8 weeks from the 25th July. This coincided with temperatures of 20°C. The use of resistant varieties alongside hard de-leafing practices, combined with thorough clean-up methods has meant that this nursery achieved good control of the disease even when conditions were favourable for disease epidemics.

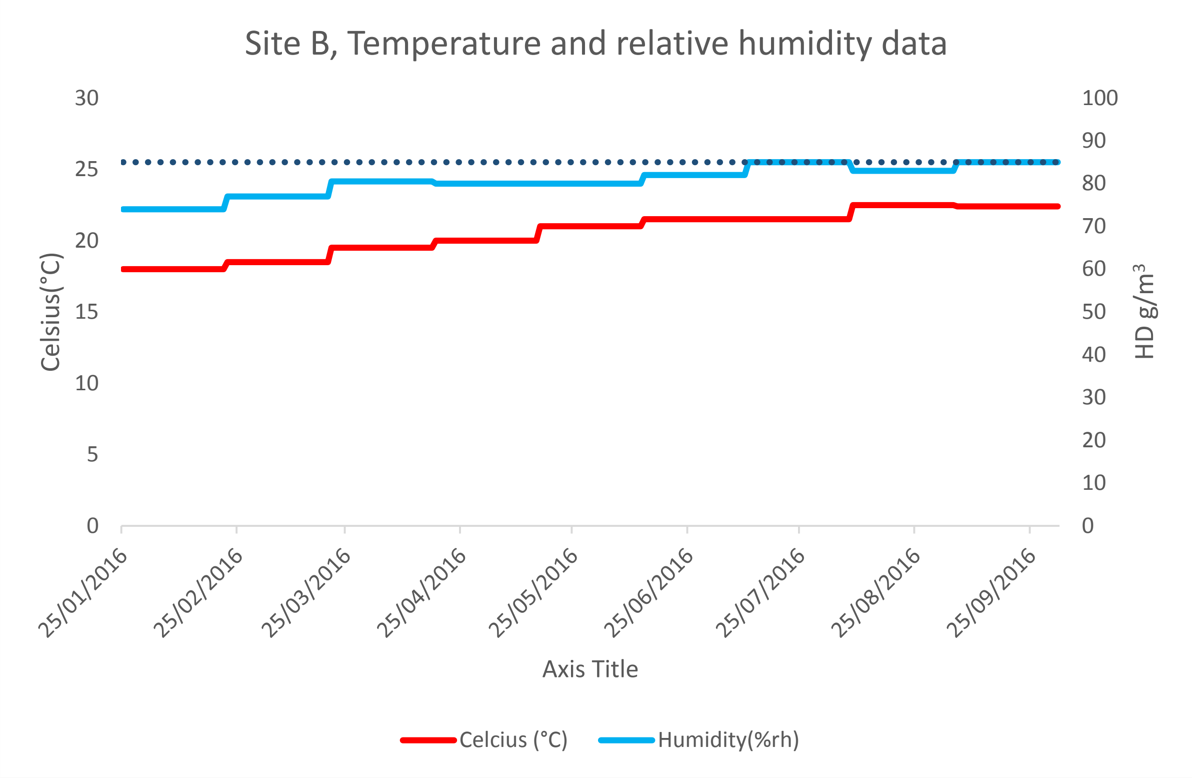

Figure 10 Average monthly temperature and humidity (RH) conditions at Site B. Dotted lines on each graph denote the HD values provided by two growers which were reported to give good disease control (2.8 and 3.5g/m^3).

Tomato leaf mould was first seen at Site C in June 2016 but was not assessed until September. Environmental data was supplied from January through to mid-November for Houses 1 and 2 (both 12 years old) which contained the resistant varieties Piccolo and Avalantino respectively. The exact date the disease was first seen is unknown, but it was seen in June. Figures 11 and 12 show the environmental conditions from mid-May until the end of June to include the time period over which infection initially occurred. Environmental conditions for the entire season for houses 1 and 2 are located in Appendix 2.

Humidity data in the form of relative humidities was supplied by Site C and RH values above 85% are denoted by a dotted line on Figures 11 and 12. During this six week period high humidities were recorded in both houses with RH values exceeding 85% during 59% of the time in house 1, and 63% of the time in house 2. These humid conditions coupled with an average temperature of 21°C are near optimal for the disease to infect and spread, and it is no surprise that tomato leaf mould developed during this period. Site C reported that disease incidence was greater during 2016 than in previous years and that end of season disease severity were much higher than that seen in the past. Both Piccolo and Avalantino grown in

Houses 1 and 2 developed leaf mould even though they are claimed resistance to it. It is probable that novel *P. fulva* isolates able to overcome the existing resistance genes in Piccolo and Avalantino were present on Site C. Combining this with the near perfect conditions for the disease to grow would explain the increased disease incidence even though the grower treated for tomato leaf mould preventatively.

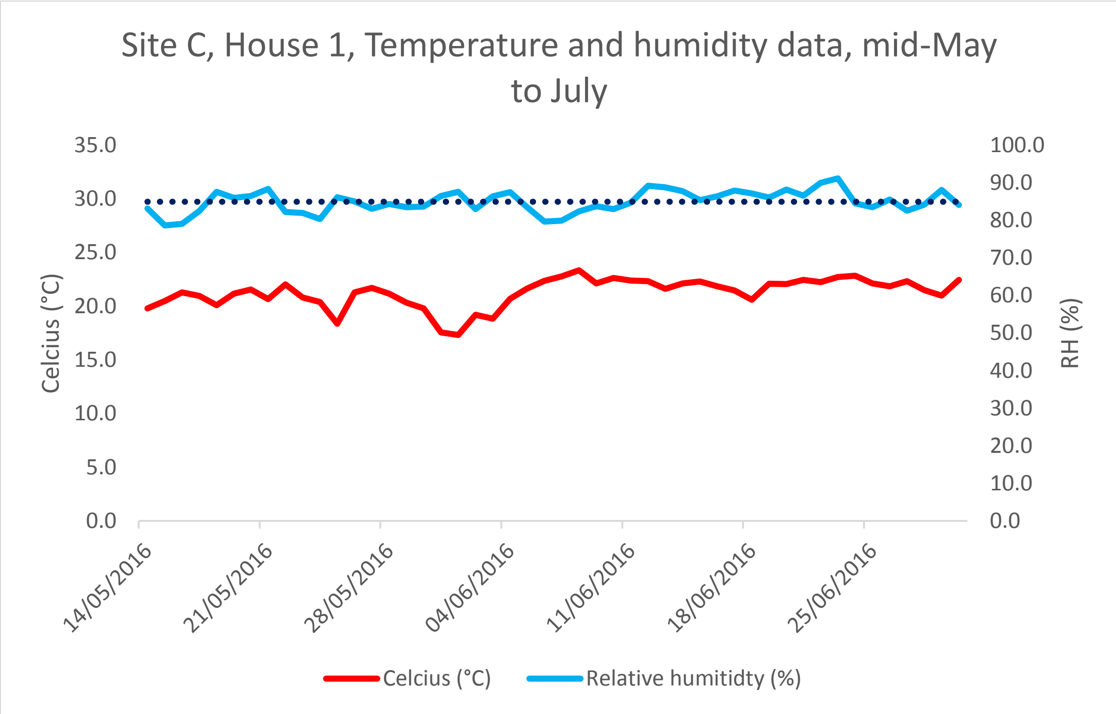

Figure 11 House 1, Site C environmental conditions encompassing the time period in which initial infection occurred (14th May – 30th June 2016). Temperatures above the dotted line at 85% relative humidity indicate conditions favourable to tomato leaf mould development.

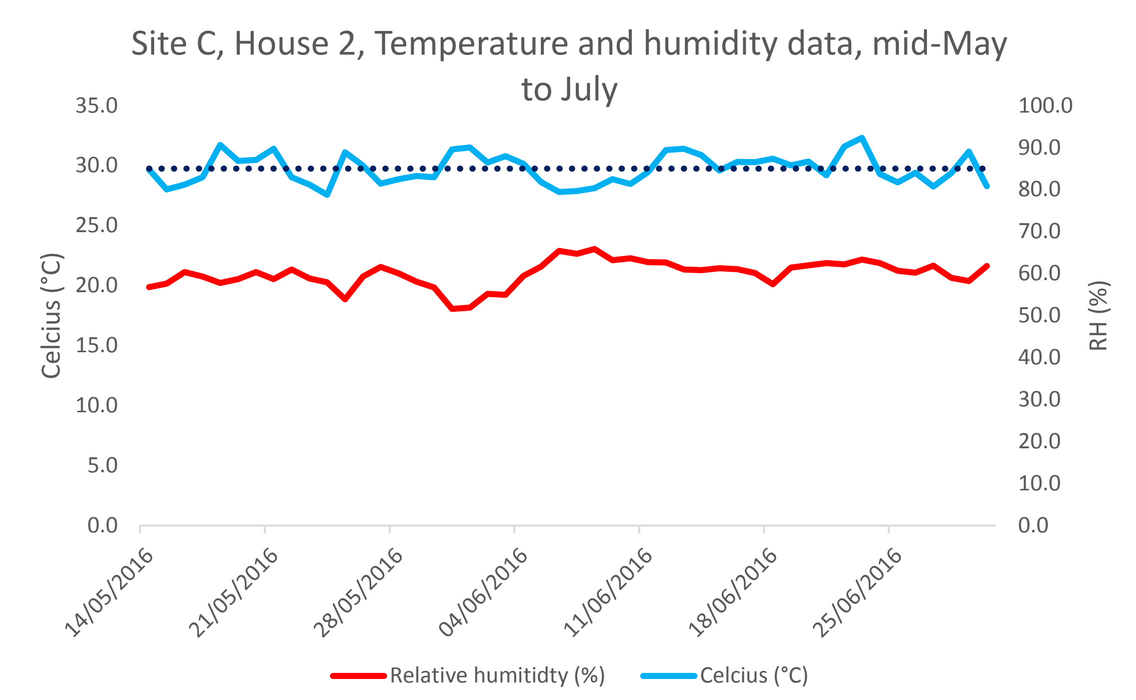

Figure 12 House 2, Site C environmental conditions encompassing the time period in which initial infection occurred (14th May – 30th June 2016) Temperatures above the dotted line at 85% relative humidity indicate conditions favourable to tomato leaf mould development.

Objective 2 - To collect isolates of *P. fulva* from affected crops and determine their sensitivity to current standard fungicides and potentially useful new products

The history of each isolate collected from five different tomato varieties from the four commercial sites visited between 2015 and 2016 is summarised in Table 7.

Table 7. Isolates collected from commercial sites in 2015 and 2016

Number	Site	Variety	Year	Spray product history
1.	A	Kierano	2015	None
2.	B	Ternetto	2016	None
3.	B	Ternetto	2016	Switch
4.	C	Sophie Jane	2016	None
5.	C	Piccolo	2016	None
6.	D	Amoroso	2016	Amistar
7.	D	Piccolo	2016	Amistar

Efficacy trial

The levels of leaf mould achieved in this trial were very low, especially in comparison to trials carried out on full plants in 2013. Low levels occurred in the untreated, making comparison difficult. No statistically significant differences were found between treatments at any of the assessment dates (Table 8). Two treatments, Reflect and Switch, appeared to result in a considerably higher level of leaf mould than in untreated plots (Figure 13). Reflect (isopyrazam) is primarily used for powdery mildew not leaf mould control and this may go some way in explaining these results. *P. fulva* antagonists may have been removed by this treatment allowing the disease to spread more than it would have naturally in the untreated. The selection of isolates without the often associated hyperparasite, *Hansfordia pulvinata*, may have changed how the pathogen interacts with the tomato leaf. In past trials Switch has been shown to treat *P. fulva* effectively, however between 2015-2016 growers reported that Switch is becoming less effective with many experiencing shorter spray intervals as a result of this. This effect may be due to natural populations of *P. fulva* developing resistance to Switch. Switch was still effective on leaves from sites where it had not been sprayed before further pointing to resistance developing. The results of the resistance testing, below, also point towards this possibility. With such a low disease incidence in the experiment further work with greater disease pressure is required to further determine product efficacy.

Table 8. Severity (% leaf area affected) of infection in a detached leaf assay – ADAS Boxworth, 2016

		Severity (% leaf area affected) d.a.i.			
Treatment	Product	9	15	17	21
1.	Untreated	0.1	0.0	0.0	0.1
2.	Amistar	0.3	1.0	0.3	0.4
3.	Switch	1.3	1.9	1.9	4.9
4.	Prolectus	0.6	1.1	1.1	1.3
5.	Reflect	1.3	1.5	1.6	1.4
6.	Mycostop	0.2	0.4	0.8	0.8
7.	Prestop	0.1	0.0	0.2	0.2
8.	Stopit	0.3	0.0	0.0	0.0
p value		0.36	0.52	0.56	0.35
LSD		1.37	2.26	2.31	4.32

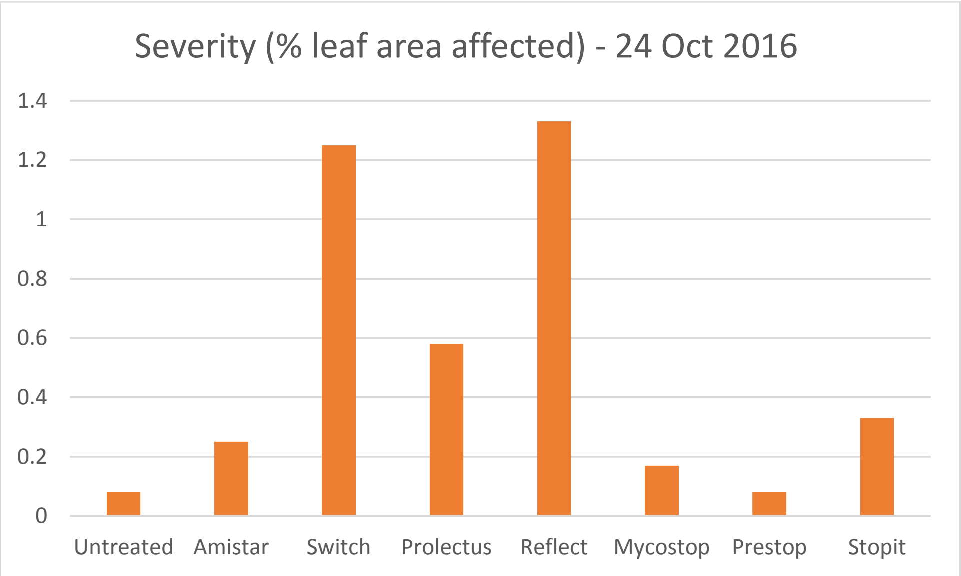

Figure 13. Mean severity of *P. fulva* by treatment at 10 days after inoculation

Resistance testing

It was requested that the resistance tests be run on detached leaves rather than agar plates due to *P. fulva* hemibiotrophic nature. It is possible that this method may have affected the way *P. fulva* responded to different fungicide products e.g. stomata may have failed to open sufficiently. *P. fulva* infects plant tissue through leaf stomata, as a biotroph, and the senescing state of the leaves may have affected infection success. Towards completion of the experiment many of the leaves showed signs of considerable deterioration. Severity was scored on leaf area visibly affected at four dates for each of the product concentrations, increasing in order of magnitudes from 0.1 to 100 ppm (Table 9). No significant differences were found for any product at any concentration. Amistar, the current industry standard appeared to perform as expected, showing good control at 10 ppm and above, whilst boscalid appeared to perform poorly even at high concentrations and even though it has previously shown to offer control. Inhibition curves for each treatment are found in Appendix 3. Results were unexpected with severity scores for many concentrations and products appearing to exceed the untreated control including those for Comet and Filan.

Table 9. The effect of different concentrations of four treatments on severity of *P. fulva* infection at four assessment dates

Treatment				Severity per plot (% leaf area affected)			
No.	Product	a.i.	Concentration (ppm)	24-Oct	28-Oct	31-Oct	03-Nov
1	Untreated	-	0	0	0	0	0.08
2	Untreated	-	0	0.25	0.33	0.33	0.42
3	Amistar	Azoxystrobin	100	0	0	0	0
4	Amistar	Azoxystrobin	10	0	0	0	0.42
5	Amistar	Azoxystrobin	1	0.33	0.33	0	0.67
6	Amistar	Azoxystrobin	0.1	0.5	0.5	0.5	0
7	Comet	Pyraclastrobin	100	0.25	0.33	0.17	0.17
8	Comet	Pyraclastrobin	10	0.17	0.5	0.75	0.75
9	Comet	Pyraclastrobin	1	0	0	0	0.25
10	Comet	Pyraclastrobin	0.1	0.5	0.83	0.83	0
11	Filan	Boscalid	100	0.92	0.58	0.58	0.67
12	Filan	Boscalid	10	1	1.33	0.5	0.25
13	Filan	Boscalid	1	0.83	0.5	0.5	0
14	Filan	Boscalid	0.1	0.75	0.75	0.75	1.17
15	Switch	Cyprodinil (+ Fludioxonil)	100	0.42	1.33	1.33	0.83
16	Switch	Cyprodinil (+ Fludioxonil)	10	1.17	1.33	1.33	0.25
17	Switch	Cyprodinil (+ Fludioxonil)	1	0	0	0	0.5
18	Switch	Cyprodinil (+ Fludioxonil)	0.1	0	0	0	0.33
p value				0.711	0.617	0.575	0.911
LSD				1.247	1.439	1.326	1.257

The three terminal leaflets of each plot were also scored separately to reduce the variation introduced by leaf deterioration. Again Amistar performed best with boscalid performing poorly. No significant differences were found between treatments or concentrations of treatments used on disease severity. These data are located in Appendix 4.

Objective 3 - To establish a best practice guide for control of leaf mould in crops based on previous research, grower experience and the results of investigations and tests conducted in this project

The findings of work under Objectives 1 and 2 have been collated, and a grower focussed best practice guide has been produced. This can be accessed via the AHDB website, and its text is included in Appendix 5.

Conclusions

- The incidence of tomato leaf mould in the UK is variable – whilst many sites never experience it, others consistently become infected each year, at least six nurseries were infected in 2016
- Where and when tomato leaf mould does occur, its severity also varies enormously – on one site almost all varieties were infected to differing degrees, whilst on another only hotspots of disease persisted for most of the season
- Amistar, found to be effective against leaf mould in project PE 018, continues to perform and offers good control
- The efficacy of Switch is reported to decline with continued use, even in subsequent years, and experimental data appears to confirm this

Knowledge and Technology Transfer

- Best practice guide: Tomato leaf mould update.
- Kaye D (2017), Tomato leaf mould update AHDB Grower (submitted).

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Appendices

Appendix 1.

Leaf mould questionnaire supplied to the six contributing growers

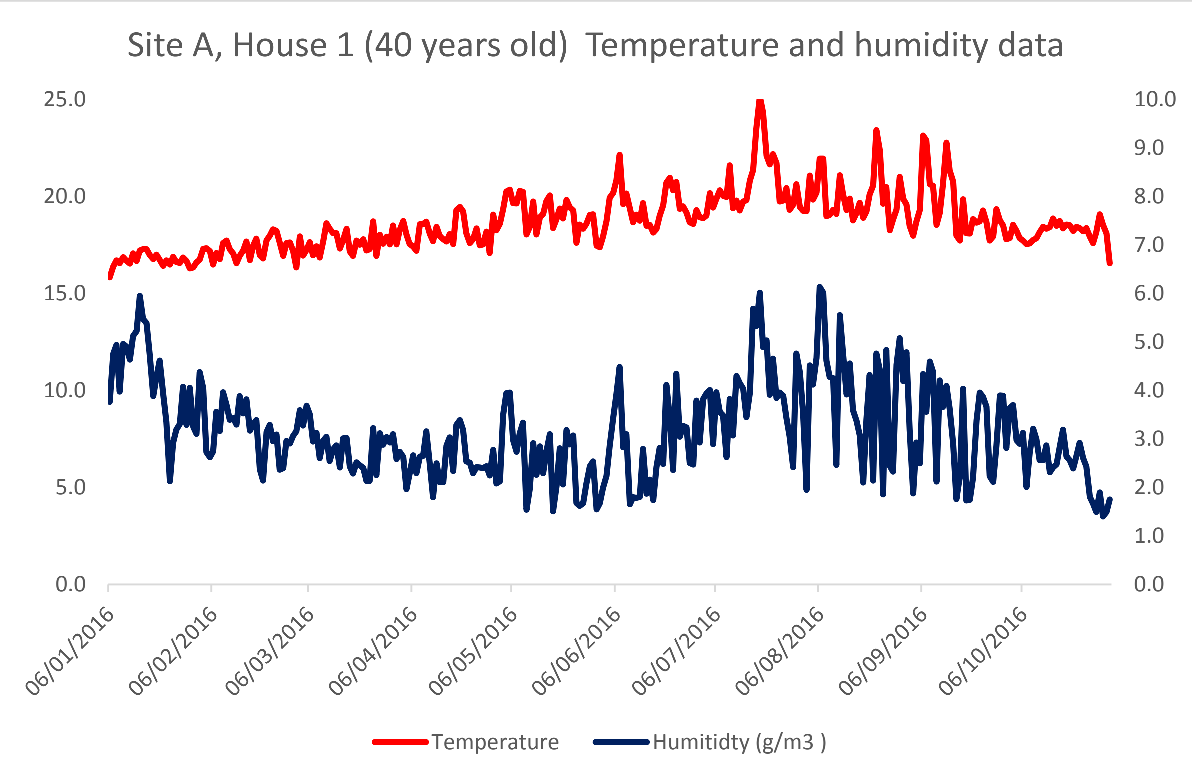
Site Details	
Visit number & date	
Grower	
Address	
Crop information	
Historical experience (vs. current)	
Spatial distribution	
Yield and other impacts	
Crop husbandry issues	
Variety	
Cultural controls:	
1.	
2.	
3.	
4.	
5.	
Chemical control	
1.	
2.	
3.	
Biological control and efficacy	
1.	
2.	
Treatment timing (first and interval)	
Spray equipment, nozzles, application, water volumes	
Crop clean-up procedure	
Changes made year on year?	
Environmental controls	
Likely gaps/weaknesses in control	
Any other comments	

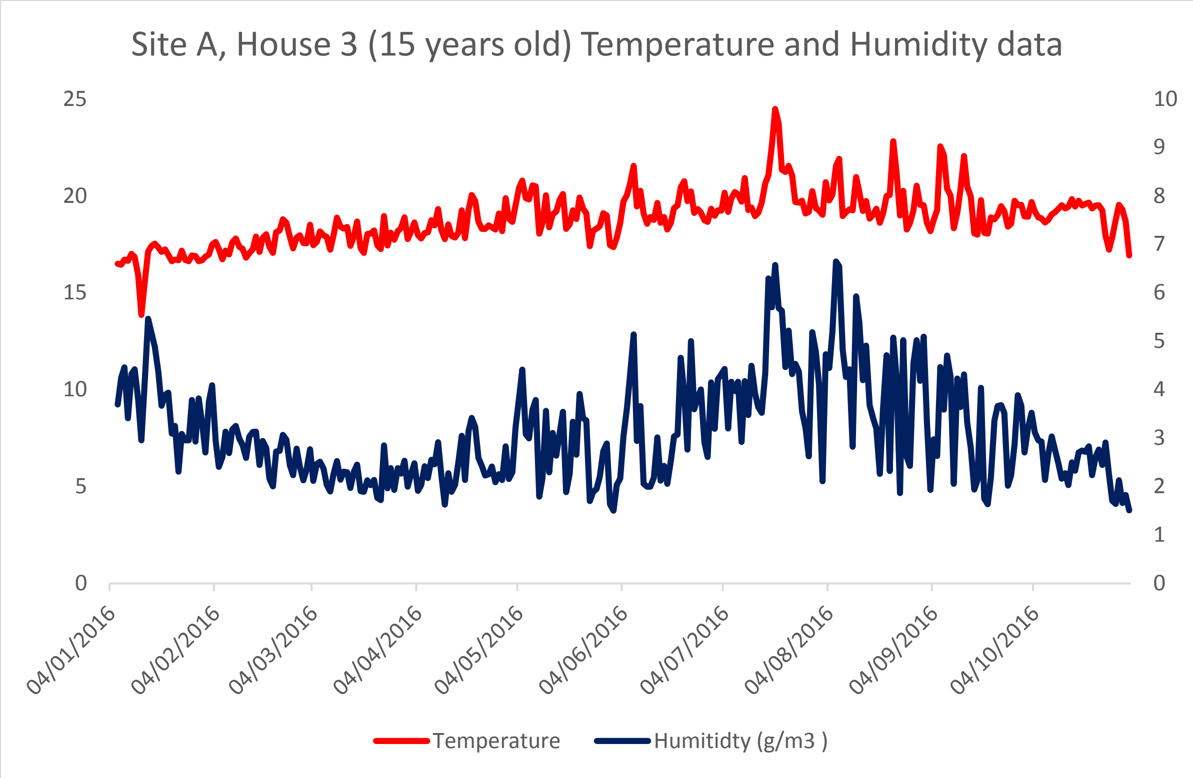
Append:

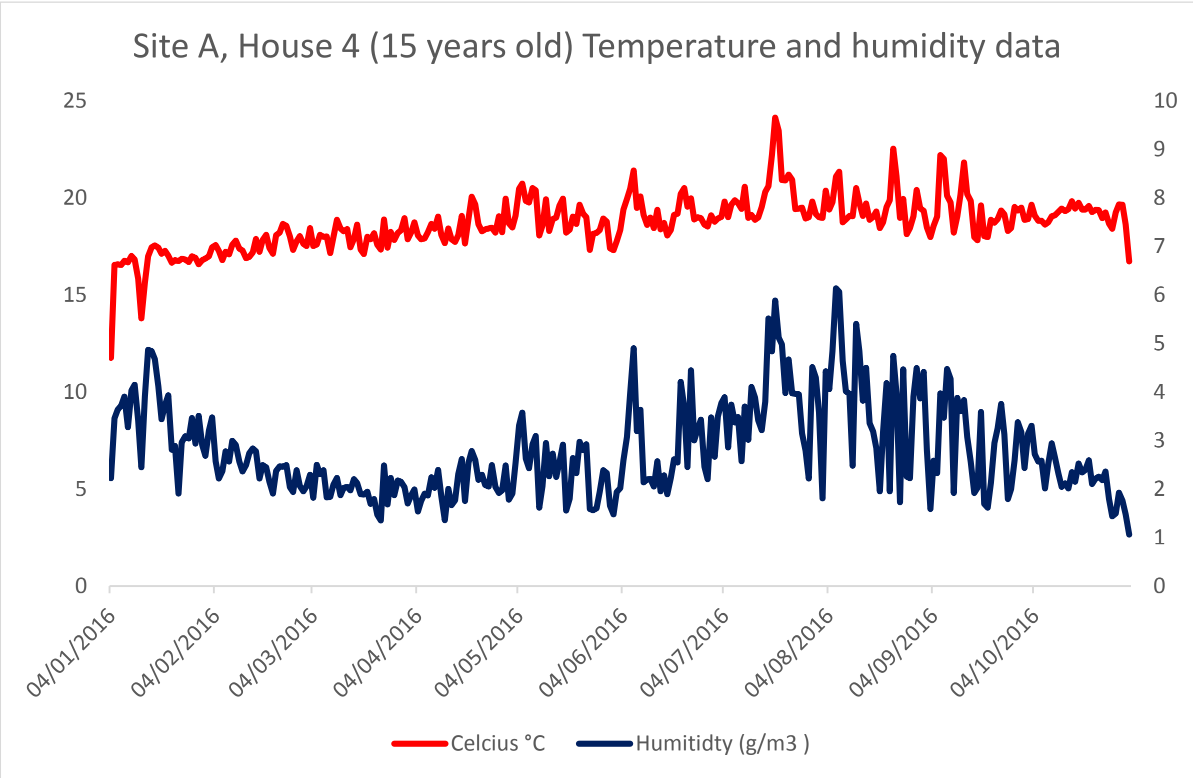
- **Environmental monitoring data**
- **Images of symptoms**
- **Spray records**
- **Symptom assessment – assess a minimum of 10 plants in 10 rows (sample below)**

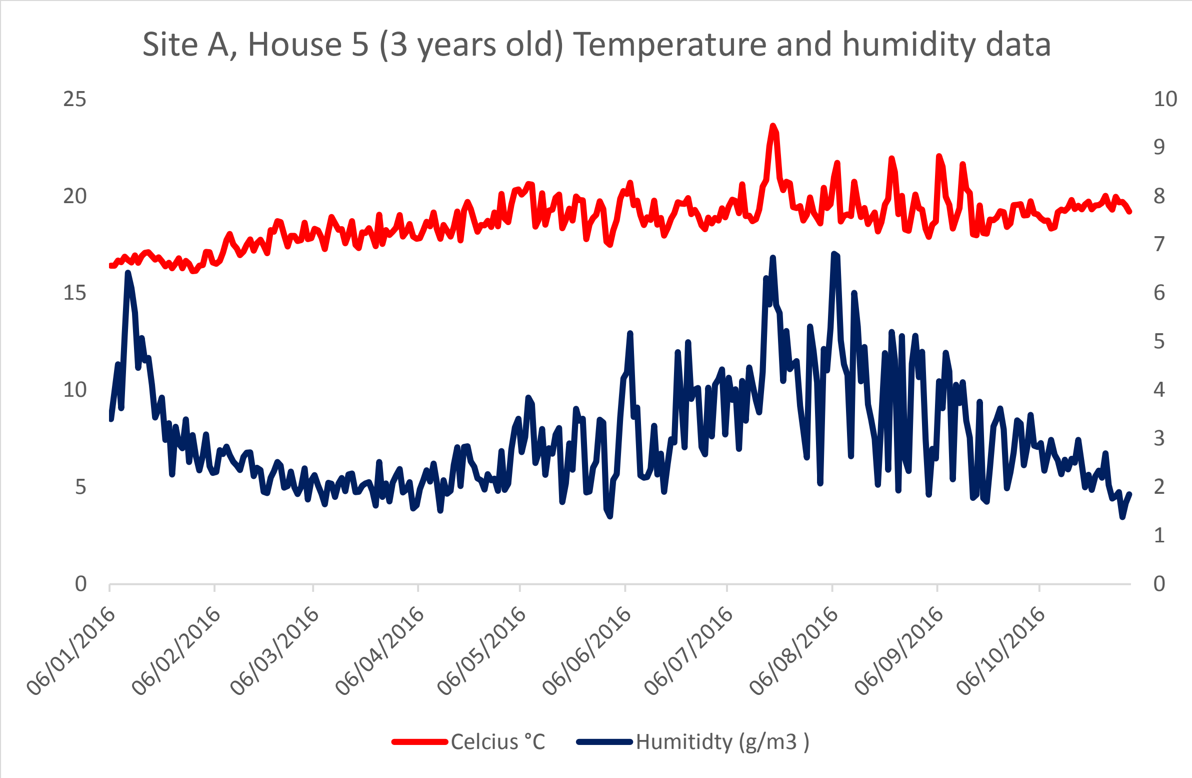
Date	
Visit	

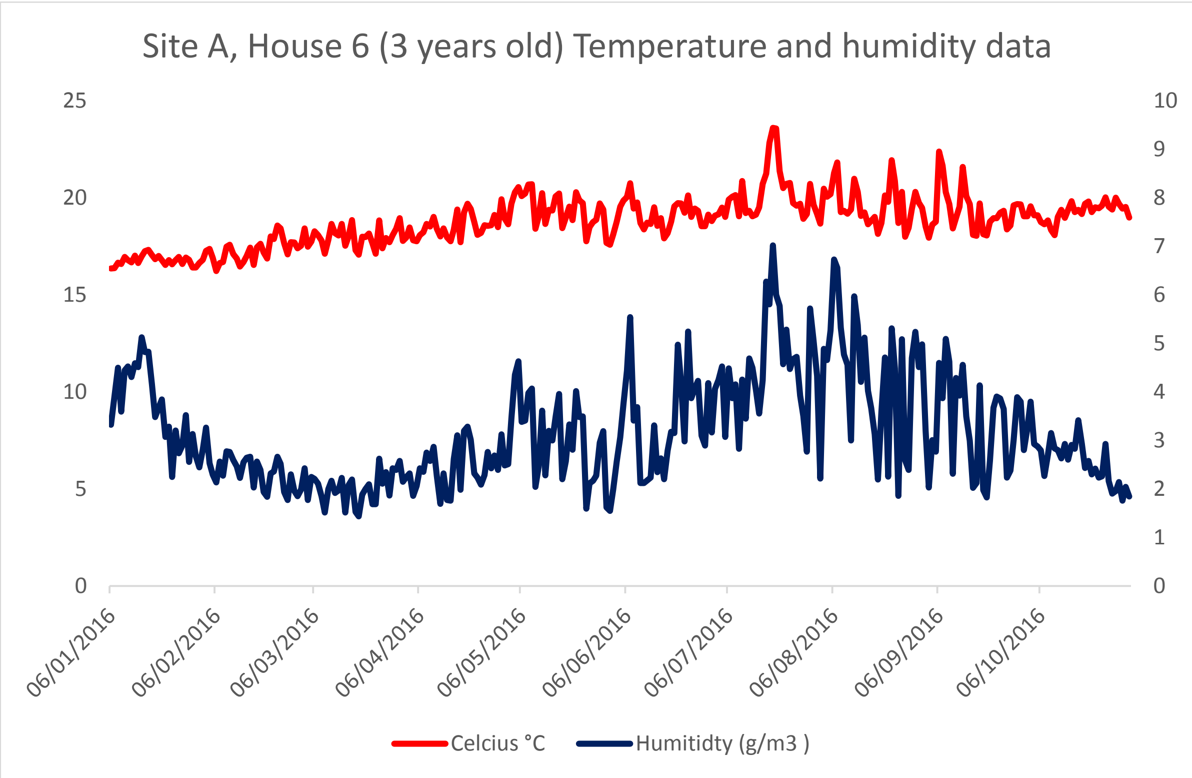
Appendix 2: Environmental conditions at Site A and Site C during the entire season.

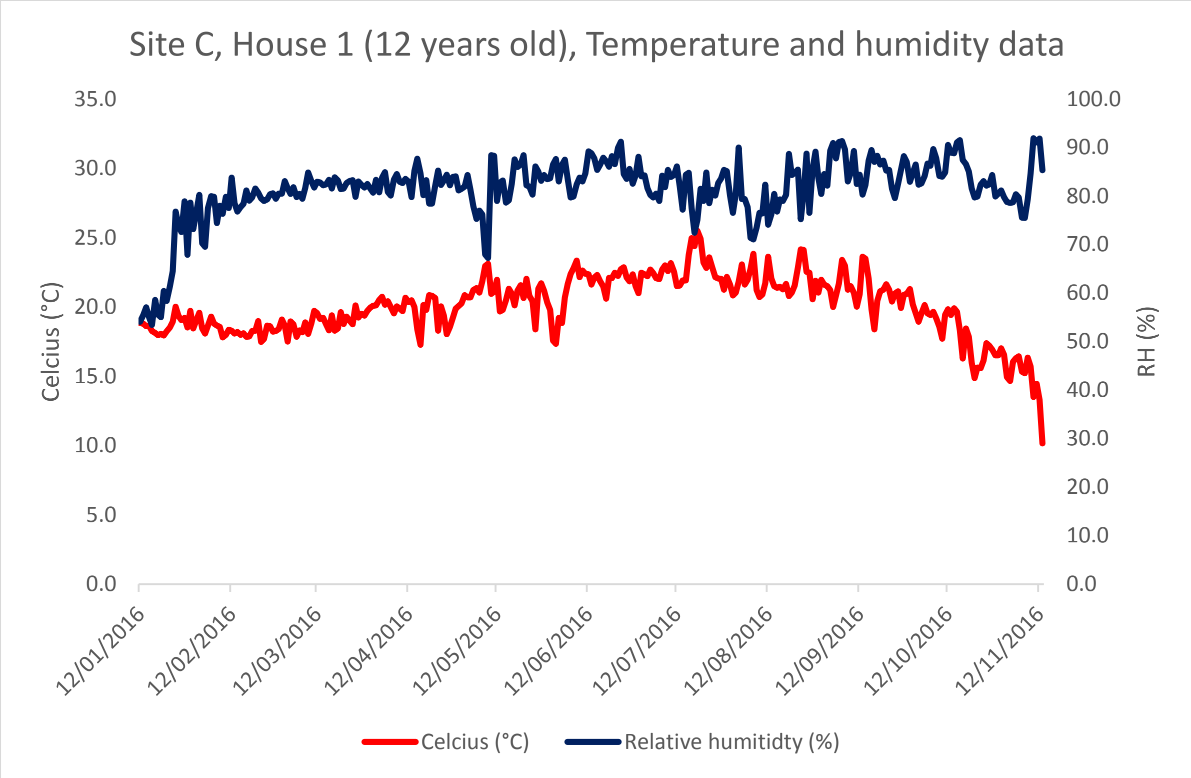


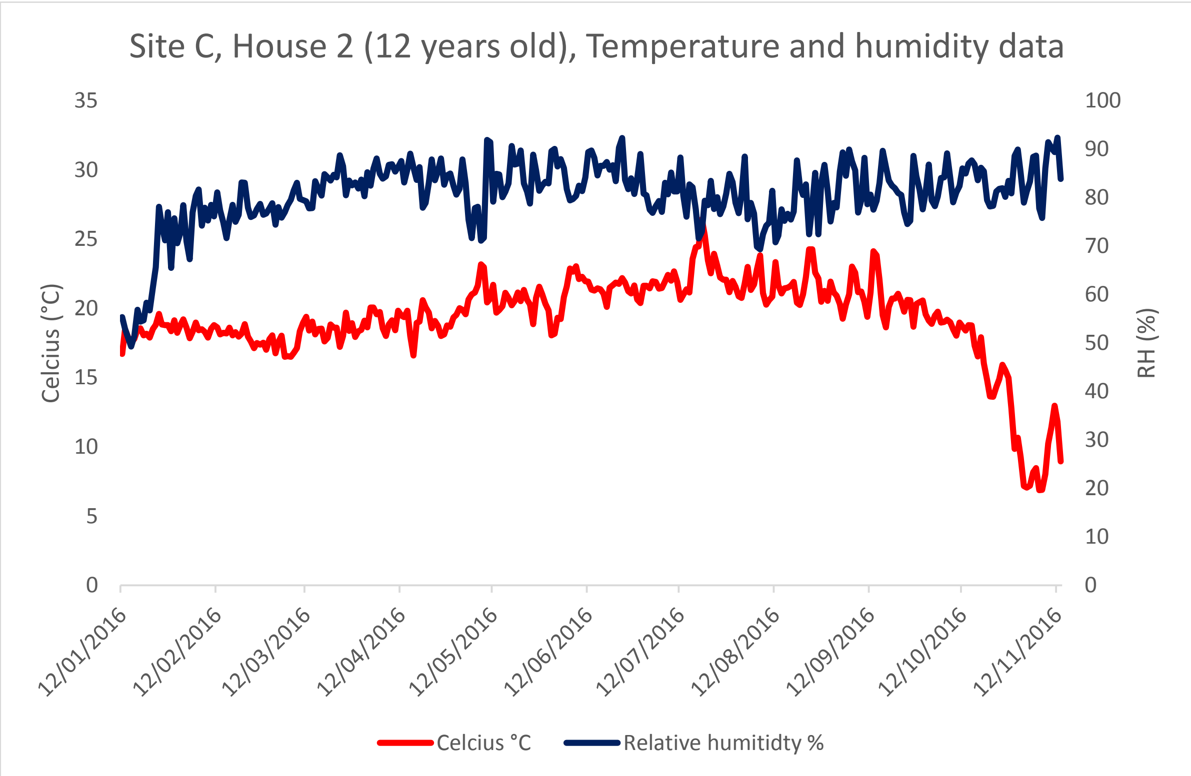

Site A, House 3 (15 years old) Temperature and Humidity data
25
20
15
10
5
0
10
9
8
7
6
5
4
3
2
1
0
04/01/2016
04/02/2016
04/03/2016
04/04/2016
04/05/2016
04/06/2016
04/07/2016
04/08/2016
04/09/2016
04/10/2016
Temperature
Humitidty (g/m3)


Site A, House 4 (15 years old) Temperature and humidity data
25
20
15
10
5
0
10
9
8
7
6
5
4
3
2
1
0
04/01/2016
04/02/2016
04/03/2016
04/04/2016
04/05/2016
04/06/2016
04/07/2016
04/08/2016
04/09/2016
04/10/2016
Celcius °C
Humitidty (g/m3)


Site A, House 5 (3 years old) Temperature and humidity data
25
20
15
10
5
0
10
9
8
7
6
5
4
3
2
1
0
06/01/2016
06/02/2016
06/03/2016
06/04/2016
06/05/2016
06/06/2016
06/07/2016
06/08/2016
06/09/2016
06/10/2016
Celcius °C
Humitidty (g/m3)

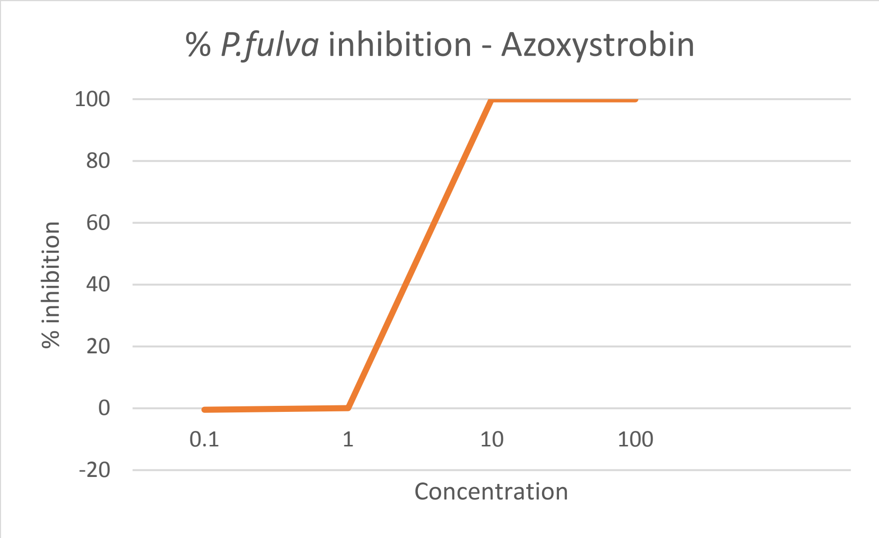

Site A, House 6 (3 years old) Temperature and humidity data
25
20
15
10
5
0
10
9
8
7
6
5
4
3
2
1
0
06/01/2016
06/02/2016
06/03/2016
06/04/2016
06/05/2016
06/06/2016
06/07/2016
06/08/2016
06/09/2016
06/10/2016
Celcius °C
Humitidty (g/m3)



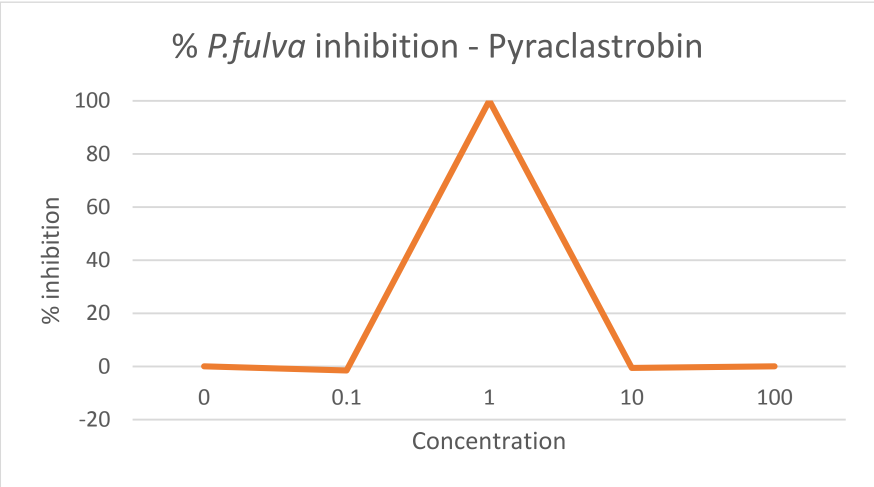


Appendix 3. Percent inhibition of *P. fulva* by four fungicides, azoxystrobin, pyraclastrobin, boscalid and cyprodinil (and fludioxonil) against concentrations, 0, 0.1, 1, 10 and 100 ppm of

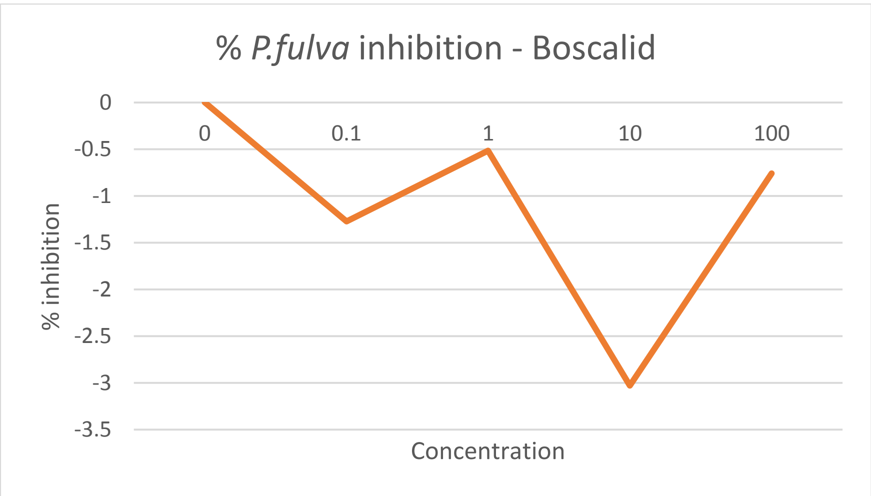
a.i.



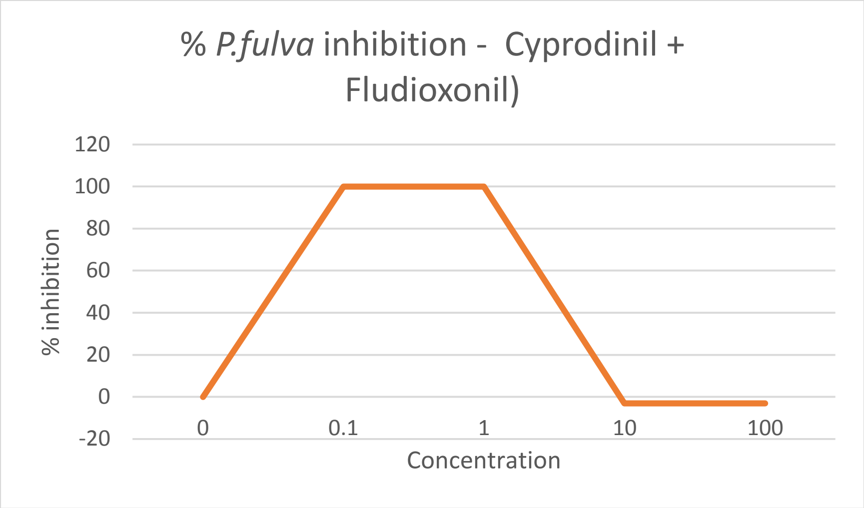
% *P.fulva* inhibition - Azoxystrobin
100
80
60
40
20
0
-20
% inhibition
0.1
1
10
100
Concentration



% *P.fulva* inhibition - Pyraclastrobin
100
80
60
40
20
0
-20
% inhibition
0
0.1
1
10
100
Concentration



% *P.fulva* inhibition - Boscalid
0
-0.5
-1
-1.5
-2
-2.5
-3
-3.5
% inhibition
0
0.1
1
10
100
Concentration


% *P.fulva* inhibition - Cyprodinil + Fludioxonil)
% inhibition
120
100
80
60
40
20
0
-20
0
0.1
1
10
100
Concentration

Appendix 4

The effect of different concentrations of four treatments on severity of *P. fulva* infection on three terminal leaves at four assessment dates

				Severity per three terminal leaves (% leaf area affected)			
Treatment	Product	a.i.	Concentration (ppm)	24-Oct	28-Oct	31-Oct	03-Nov
1	Untreated	-	0	0	0	0	0.17
2	Untreated	-	0	0.5	0.75	0.75	1
3	Amistar	Azoxystrobin	100	0	0	0	0
4	Amistar	Azoxystrobin	10	0	0	0	1.08
5	Amistar	Azoxystrobin	1	0.67	0.67	0	0
6	Amistar	Azoxystrobin	0.1	0.83	0.83	0.83	0
7	(Comet)	Pyraclastrobin	100	0.5	0.58	0.33	0.33
8	(Comet)	Pyraclastrobin	10	0.5	1.17	1.5	1.5
9	(Comet)	Pyraclastrobin	1	0	0	0	0.67
10	(Comet)	Pyraclastrobin	0.1	2.33	1.58	1.58	0
11	(Filan)	Boscalid	100	2	1	1	1.67
12	(Filan)	Boscalid	10	1.33	1.67	0.83	0.33
13	(Filan)	Boscalid	1	1.67	1.17	0.83	0
14	(Filan)	Boscalid	0.1	1.33	1.33	1.33	1.67
15	Switch	Cyprodinil (+ Fludioxonil)	100	1.17	2.67	2.67	1.67
16	Switch	Cyprodinil (+ Fludioxonil)	10	2.17	2.5	2.5	0.67
17	Switch	Cyprodinil (+ Fludioxonil)	1	0	0	0	0.83
18	Switch	Cyprodinil (+ Fludioxonil)	0.1	0	0	0	1.17
p value				0.483	0.655	0.508	0.812
LSD				2.303	2.606	2.459	2.172

Appendix 5. Tomato leaf mould best practice guide.

Best Practice Guide: Tomato leaf mould

Sarah Mayne and Dave Kaye, RSK ADAS

Background

Tomato leaf mould is one of the most destructive foliar diseases of tomato when the crop is grown under humid conditions. It has recently reappeared in some UK crops, and on a few nurseries has persisted overwinter between one crop and the next. The disease affects both conventional and organic crops. In this best practice guide information is provided on disease symptoms and epidemiology, and provides guidance on how to manage the disease using cultural and chemical measures, effective crop husbandry, varietal resistance and plant protection products.

The pathogen

Tomato leaf mould caused by *Passalora fulva* (previously known as *Cladosporium fulvum*) is a destructive foliar disease of increasing importance in the UK. Outbreaks have occurred most years since 2000 and affected a range of varieties. Previously well controlled by genetic resistance, new outbreaks appear to be caused by the cultivation of varieties without claimed resistance and the emergence of strains capable of overcoming resistance genes deployed in current varieties. There is no available easy method in place to identify strains apart from the classical approach determining pathogenicity, one isolate at a time, which is time-consuming and costly.

The fungus has been found both on and within the seed coat, and seed borne infection has been reported. Infected seed planted in sterile compost has been shown to develop *P. fulva* symptoms on cotyledons. This indicates the disease can be present from sowing and this should be considered when developing treatment strategies.

P. fulva produces only one spore type, the conidium. Produced in vast numbers these spores are easily spread by air currents, insects and via hands and clothing. Conidia are highly resistant to low temperatures and dry conditions. It is believed that spores survive on surfaces from one crop to the next. Although the pathogen adopts a biotrophic lifestyle on living tissue, the pathogen can also survive for long periods on dried leaf debris saprophytically.

Figure 1. Early symptoms of yellow spotting on the upper leaf surface.

Figure 2. Typical disease symptoms of velvety brown mould growth on the underside of the leaf

Environmental conditions strongly influence *P. fulva* incidence and severity. High humidity is very favourable with relative humidities of 85% or above critical, allowing spores to germinate and runner hyphae to elongate and penetrate the stomata. Warm temperatures of 22-24^{o}C combined with high humidities lead to *P. fulva* epidemics. At temperatures of 12^{o}C or lower the disease will not usually be an issue but the disease is still able to grow at temperatures as low as 4^{o}C. After 7 days diffuse yellowish spots appear on the leaf surface (Figure 1), with advanced stages showing aggregations of fungal hyphae from stomata exiting the leaf releasing large numbers of conidia, appearing as a brown mould on the underside of the leaf surface (figure 2).

No sexual stage of *P. fulva* has been observed. Although asexual the fungus mutates rapidly and has a short life-cycle, this has led to the development of several new races able to overcome existing resistance genes. Several different races have been described with many resistance genes identified.

Control

Timing is key

The importance of timing cannot be overstated. Disease symptoms usually present in April onwards so monitor the crop from this time. If an infection is likely to occur preventative measures should be implemented early to prevent disease establishment or to mitigate disease affects. Preventative measures include both cultural and chemical control. Tomato leaf mould can result in significant losses to growers. Poor control can lead to *P. fulva* epidemics which decrease yields and has led to the removal of crops several weeks early. Significant spore numbers present in the environment can also cause human health issues of the eyes and lungs leading to sickness or the need for additional PPE when working in the crop.

Environmental control

Prevention of disease by managing the glasshouse environment is much easier than managing the disease. Good environmental management will reduce incidence and severity of tomato leaf mould and has the potential to control the disease to levels in which no fungicide sprays are required. Glasshouses with relative humidities (RH) optimal for *P. fulva* (≥85%) are at the greatest risk. It is essential that the crop is well ventilated and relative humidity maintained as low as feasible with periods of humidity above 85% minimised. On sites with less severe infection, the disease tends to breakout in humid microclimate 'hotspots', e.g. where there are leaks and pooling water, or where condensation occurs at the edge of glasshouses. The disease will often appear in these same locations year on year so address and monitor these sites as soon as they are found.

The current practice of humidity control in edible crops is generally based on humidity deficit (HD). The use of HD allows optimised plant development and growth through promotion of transpiration. Relative humidity gives a better indication of the risk of condensation developing, and therefore in terms of fungal growth and disease RH is more relevant than HD. Dutch growers and some UK nurseries are now using RH as well as HD to achieve both good yields and control disease. Growers using HD should consider routinely checking RH levels, especially on the lower leaves. Nurseries with computerised systems which automatically controls humidity below 85% RH are less likely to experience issues than those without.

Disease levels at several sites using a variety of glass ages were monitored during 2016. The age and condition of glass was found to have an impact on tomato leaf mould. Generally old glass is leakier than new, creating favourable conditions for leaf mould development. A grower reported in 2016 that their site's initial *P. fulva* outbreak occurred in a glasshouse with old, low glass before spreading to the other houses. Many houses with middle-aged glass exhibited disease levels similar to those with old glass than new, indicating the benefits of new glass could be short lived. Growers with new glass should avoid becoming complacent, one grower reported greater leaf mould in new glass than old. New glass can create a more sealed environment resulting in higher humidity conditions, whereas old glass is often more ventilated. One site which suffered leaf mould infection in 2015 had no infection in 2016 despite growing organic crops in old, widespan glasshouses. Glass condition is an important factor in the likelihood of disease establishment but its impact is influenced by how each grower manages the crop, the environment and the quality of end of season clean-up/disinfection.

The recent development of the Dutch "New way of growing" for protected edibles enables substantial energy savings through reduced heating at certain times of day and the increased use of screens. This growing technique can save considerable sums of money in energy bills but can result in a more humid growing environment. If a foliar disease becomes established, the associated disease management practices, combined with any reduction in yield potential could mean these savings are offset. A balanced management strategy needs to be developed to encompass all these factors minimising disease risk whilst reducing overheads.

Hygiene

During 2016 sites that implemented good hygiene and clean-up protocols still experienced infection to varying extents. *P. fulva* spores are very resistant to dry conditions and are believed to survive in a dormant state from one crop to the next or live saprophytically on dry debris making complete disinfestation challenging. Only one organic site was completely successful in eradicating the disease. This was in part due to low disease incidence during the previous season combined with a comprehensive clean-up operation.

Figure 3. *P. fulva* can survive saprophytically on plant debris between crops and restart the infection cycle if not removed

Growers should be aware that using certain types of sheet weed suppressants e.g. Mypex which allow soil and dust through the floor could re-introduce infection to glasshouses and serve to create a humid microclimate. Some sites use a plastic floor covering, replaced each year, to act as a physical barrier preventing introduction of soil borne spores and moisture into the crop and have had good levels of disease control. Growers should be wary of using impermeable plastic sheeting in areas where leaky equipment may result in pooled water forming.

Throughout the year it is important to maintain high standards of glasshouse and crop hygiene measures, including washing and disinfecting hands during movement between glasshouses. Crops should be monitored routinely for disease, especially at known hot-spots and high risk areas and actions should be taken before the disease becomes epidemic.

Effective crop clean-up and glasshouse disinfection can be key to lowering the amount of viable inoculum present to infect next year's crop and all remaining plant debris must be removed from the glasshouse (Figure 3). Sites which have experienced severe disease levels will face more of a challenge in completely eliminating all *P. fulva*. All equipment including irrigation lines and pegs needs to be disinfected and all areas of the glasshouse structure treated, misting where appropriate. Disinfectant products are most effective when used at their full recommended rates, for as long as possible. The most effective products against *P.*

fulva, such as Hortisept Pro, Unifect-G and Menno Florades (PE 018) should be used to ensure clean-up investment and labour is most cost-effective.

Chemical control

Fungicides

Previous work on biological and fungicidal control of tomato leaf mould in PE 018 and responses from grower questionnaires confirmed that Amistar can be considered the current industry standard, followed, by Switch. *P. fulva* infects via stomata and lives as a biotroph within the apoplast, chemical fungicides with translaminar or systemic action, such as Amistar, provide the most effective treatment option. Treatments of these types will also allow for some errors in terms of coverage. Excellent coverage will be essential when using purely contact acting treatments. Though less effective than Amistar and Switch, the use of other products such as Signum and Teldor should be incorporated into spray programs, due to limitations on application numbers/maximum dose limits, as part of an integrated management strategy.

Biofungicides

A few biofungicides are now approved for use on tomato in the UK, with several more in development for registration. PE 018 found Serenade ASO to be the most effective biofungicide tested, though it was not as effective as conventional products. The use of a biofungicide preventatively can delay the need for the first fungicide treatment, minimising the number of fungicide sprays or extend spray timing intervals. One grower reported that without the availability of Serenade ASO to extend spray intervals they would have experienced significant disease problems. Biofungicides need to be used preventatively but when used effectively have the capacity to have good effects on controlling several diseases including tomato leaf mould, powdery mildew and botrytis. Restricted numbers of fungicide treatment applications, combined with developing resistance and the removal of existing actives means the use of biofungicides such as Serenade ASO is likely to become an important component of the *P. fulva* treatment program.

Resistance management

The combination of a fast life-cycle and rapid mutation rate makes the development of resistance of *P. fulva* to fungicides a concern. Strategies should integrate cultural practices which optimise fungicide use, including using different fungicide groups to minimise the risk of selecting for fungicide resistant strains. The fungicide Switch utilises two actives, fludioxonil and cyprodinil. Trial work in the past has shown Switch to be very effective, however some growers reported that Switch became less effective between the 2015 and 2016 season leading to shorter spray intervals. If confirmed this is worrying as Switch can be used a maximum of 3 times per crop, and is often used later on in the season. A reduction in *P. fulva* sensitivity to Switch would put more pressure on Amistar and other products increasing the chance of further resistance developing. Unexpected lab results in PE 030 indicate that *P. fulva* may be able to develop resistance to Switch quickly. FRAC has reported that resistance to fludioxonil and cyprodinil has been seen in other fungi.

Spray application

The industry uses a variety of different spray equipment from manual ripa sprayers to fully automatic robotic systems. Spray volumes between 1250 – 2500 L/ha are used and nozzle types include flat fan and hollow cone spray tips.

The disease is generally only found on the lower leaves and standard practice is to angle nozzles upwards towards the infection point of the stomata which are located mainly on the underside of leaves. Many growers choose to target the new growth at the top of the plant, however it is better to treat the whole plant if good coverage can be achieved. This can lead to suppression of sporulation on infected lower leaves whilst still protecting the new growth at the top of the plant. It is important to check equipment and the method of application to ensure the most effective coverage possible is achieved. Information on suggested fungicide application and rates can be found in HDC Factsheet 09/13.

Crop husbandry

Effective crop husbandry can dramatically decrease incidence and progression of *P. fulva*. The lower leaves where conditions are more conducive to the disease and where it is most commonly found can be removed. Some growers removed several leaves higher up the plant and reported very good disease control as a result, likely from a combination of reduced inoculum and decreased humidity through improved air flow. It is critical that de-leafed infected material is removed from glasshouses as this will act as a source of inoculum to re-start the infection cycle. Removed material should be placed in a covered skip to avoid spores spreading and aerial infection occurring between glasshouses.

Location of variety should be carefully considered at sites with a history of leaf mould occurrence. Growers should avoid placing susceptible varieties in areas which have had recent past infections and where spores may have persisted, and place resistant varieties in these locations instead. A grower reported that crop rotation in this fashion made for the greatest difference year on year to leaf mould occurrence at their site.

Resistant strains

As previously detailed in factsheet 09/13, genetic resistance to *P. fulva* works on a gene-for-gene basis. Most commercial varieties list their claimed resistance, or lack of, to *P. fulva*, and this is expressed as 'A-E', where strains are grouped (Table 1)

Table 1. Race groups of tomato leaf mould (A-E) and their ability to overcome five *Cf* resistance genes.

Leaf mould race group	Tomato resistance genes				
	Cf-1	*Cf-2*	*Cf-3*	*Cf-4*	*Cf-5*
A	S	S	S	R	R
B	(S)	R	(S)	S	R
C	(S)	S	(S)	S	R
D	R	R	R	R	S
E	R	S	R	S	S
S - susceptible interaction; (S) - race group can sometimes overcome this resistance gene; R - resistant interaction					

New strains of the pathogen have recently been identified in Japan, China, Korea and Poland where no varietal resistance currently exists. There is a concern that these strains will spread further west to the Netherlands and the UK. One nursery reported every variety grown except Avalantino showed infection with *P. fulva* to differing degrees. Avalantino does claim resistance to A-E, but several of the other varieties grown also did. This indicates Avalantino contains additional resistance genes not claimed by the breeder which may offer hope for the future. Table 2 lists varieties which were reported to have been infected with tomato leaf mould by growers questioned over 2015-2016, the highlighted varieties currently claim resistance to *P. fulva* groups A-E.

Table 2. Tomato varieties reported by growers as infected with *P. fulva* in 2015 and/or 2016

Variety	Breeder	Listed resistance to *Passalora fulva*
Amoroso	Rijk Zwaan	FfA-E
Angelle	Syngenta / S&G	None
Avalantino	Enza Zaden	FfA-E
Bamano	Syngenta / S&G	None
Campari	Enza Zaden	None
Garincha	Enza Zaden	None
Juanita	Monsanto / De Ruiter	None
Kierano	De ruiter	FfA-E
Lipso	Clause	None
Papeletto	Rijk Zwaan	None
Piccolo	Gaultier	FfA-E
Solarino	Rijk Zwaan	None
Sunstream	Enza Zaden	None
Sweetelle	Syngenta / S&G	FfA-E
Ternetto	Rilk Zwaan	None

If you know *P. fulva* infection is likely to occur and have struggled to control the disease in the past, growing a variety that does not claim any resistance would be unwise. It is important where possible to grow varieties with known resistance to *P. fulva*. Regardless of the varieties grown, all the topics discussed in this grower's guide should be addressed and an effective plan put in place to minimise risk of tomato leaf mould infection and to limit its spread.

Acknowledgements

We are grateful to the growers who provided answers to the growers' questionnaire and additional information they provided on control of tomato leaf mould during 2015-2016.

Further information

HDC Factsheet 09/13. Tomato leaf mould

AHDB Report PE 018: Efficacy of conventional fungicides, biofungicides and disinfectants against tomato leaf mould (*Passalora fulva*).

AHDB Report PE 030: An investigation of the current status of tomato leaf mould on UK nurseries: occurrence, disease management and potential for improved control.