Project title:	Efficacy of conventional fungicides, biofungicides and disinfectants against tomato leaf mould (*Passalora fulva*)
Project number:	PE 018
Project leader:	Dr Tim O'Neill, ADAS
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Location of project:	ADAS Boxworth, Cambs
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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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GROWER SUMMARY

Headline

- Reliable development of tomato leaf mould in experimental systems required high levels of inoculum and prolonged periods (96 h) of high humidity
- Effective conventional fungicides for control of tomato leaf mould include Amistar, Switch, Signum and Teldor, with treatment timing key to reducing disease
- The biological fungicide Serenade ASO also had good efficacy when used preventatively and under low disease pressure
- A range of disinfectant products were effective against both the spores and mycelium of *Passalora fulva*

Background

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. Amistar (azoxystrobin) has given good control in some crops, but grower reports indicate resistant strains can develop within a few years. The disease has also affected organic crops, where use of Amistar and other conventional fungicides is not permitted by the Soil Association. No plant protection products currently permitted on tomato carry a label recommendation for leaf mould control. 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. There is little information on the relative effectiveness of different disinfectants in reducing inoculum of *P.fulva*. The aim of this study was to provide tools for improved management of tomato leaf mould in both conventional and organic crops through identification of effective conventional fungicide and biofungicide treatments for use in crops, and of disinfectant treatments for use between crops.

Specific objectives of the project were:

1. To develop a controlled infection technique on tomato seedlings with *P.fulva*
2. To determine the efficacy of selected conventional fungicides and biofungicides applied as protectant and curative spray treatments for control of tomato leaf mould.
3. To determine the effectiveness of selected disinfectants for reduction of *P.fulva*

inoculum on surfaces and in debris.

Summary

Objective 1 – Development of controlled infection technique

In Experiment 1, a range of spore concentrations and periods of high humidity were applied to tomato plants cv. Gardener's Delight, a variety with no claimed genetic resistance to *P. fulva*. *P. fulva*, isolated from a crop in early 2014, was cultured on agar, and a spore suspension was used to inoculate the lower surface of tomato plant leaflets. The high concentration was 1 x 10^5 spores/ml, and the low concentration was an order of magnitude lower at 1 x 10^4. Results showed that disease development was greatest with the high level of inoculum, and an extended period (96 hours) of high humidity (Table 1 and Figure 1).

Table 1. Effect of inoculation rate and humidity period on the development of leaf mould in glasshouse tomato cv. Gardener's Delight – ADAS Boxworth, 2014 (Experiment 1)

Treatment	Inoculation rate	Humidity period (h)	% leaf area affected at intervals after inoculation		Crop vigour (0-5 index)
			15 days	18 days	18 days
1	Uninoculated	24	0.0	0.2	4.8
2	Uninoculated	48	0.0	0.0	5.0
3	Uninoculated	96	0.0	0.0	5.0
4	Low rate	24	0.3	5.0	5.0
5	Low rate	48	1.0	4.5	4.8
6	Low rate	96	1.8	**13.8**	5.0
7	High rate	24	1.8	8.8	5.0
8	High rate	48	3.3	**15.0**	5.0
9	High rate	96	**15.0**	**35.0**	**3.5**
Probability (F value) Inoculation x humidity			<0.001	0.005	<0.001
LSD (24 d.f.)			3.55	9.31	0.62

*Values in bold are significantly different from uninoculated plants.

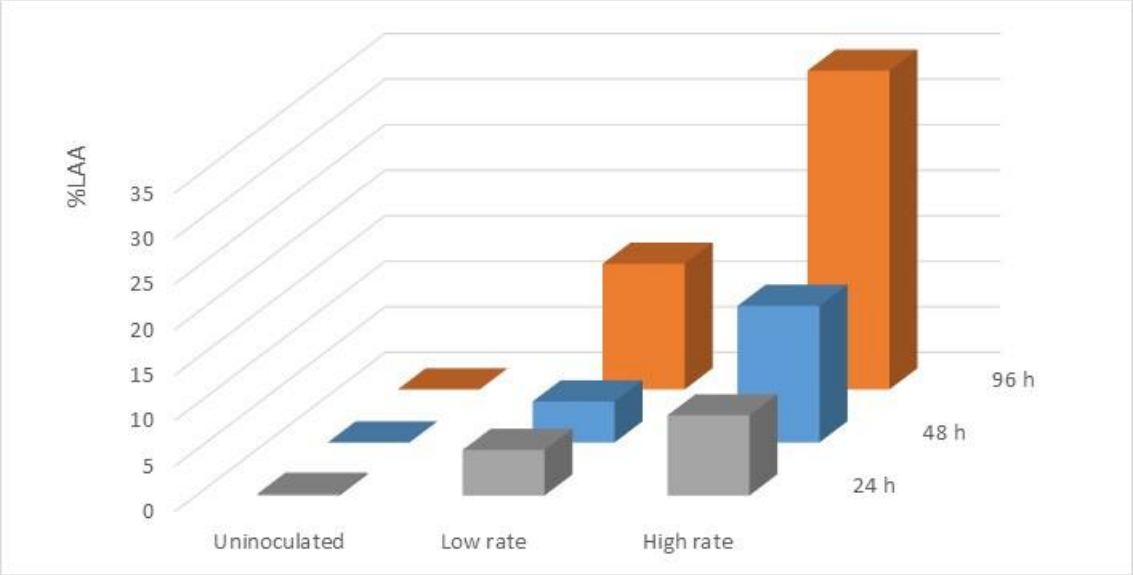

Figure 1. Effect of inoculum level and duration of high relative humidity on percentage tomato leaf area affected by tomato leaf mould as assessed 15 May, 2014 – ADAS Boxworth.

Objective 2 – Evaluation of the efficacy of conventional fungicide and biofungicide products

A total of eight products (four fungicides and four biofungicides) were tested against a water control; all of the products were approved for use on protected tomato except the coded biofungicides F185 and F186. Each product was sprayed only once, but at five different timings with reference to inoculation. These timings ranged from 5 days before to 5 days after inoculation, also including 1 day before, the day of inoculation and 1 day after. This was to establish any curative action of products against *P. fulva*, or to determine if products acted only preventatively.

Table 2. Overall effects of plant protection product and spray timing on tomato leaf mould – 2014 (Experiment 2)

Factor	Mean % plants affected		Mean % leaf area affected (Severity)	
	11 Jun	19 Jun	11 Jun	19 Jun
Product				
1. Water control	93.8 (8.4)	100 (<0.1)	1.8	4.8
2. Amistar	24.7 (6.0)	**47.3** (6.4)	**0.2**	0.9
3. Signum	46.8 (6.0)	**65.4** (5.0)	1.0	2.4
4. Switch	40.1 (7.1)	**70.4** (5.5)	**0.7**	2.7
5. Teldor	46.8 (6.9)	**73.8** (5.9)	**0.7**	1.5
6. Prestop	58.4 (6.7)	**86.4** (4.6)	1.2	2.6
7. Serenade ASO	42.7 (5.1)	**56.3** (3.8)	0.9	2.3
8. HDC F185	46.3 (6.4)	**76.1** (5.5)	0.9	1.9
9. HDC F186	42.8 (6.8)	**72.6** (5.8)	**0.7**	2.5
P value (120 d.f.)	0.143	0.002	0.033	0.142
LSD	-	-	0.9425	2.356
Timing				
-5	40.6 (5.2)	67.2 (4.0)	0.6	1.7
-1	32.9 (4.8)	58.6 (4.9)	0.4	1.3
0	36.8 (5.1)	65.6 (4.2)	0.4	2.1
1	50.0 (5.3)	72.7 (4.2)	1.0	2.0
5	58.6 (4.9)	79.0 (3.8)	**1.4**	3.4
P value (120 d.f.)	0.016	0.051	<0.001	0.005
LSD	-	-	0.9126	2.281

*Values in bold are significantly different from the water control (upper columns) or the day 0 timing (lower columns); ( ) – standard error. See Tables 11 and 12 for results of individual treatments.

Results show that whilst all treatments reduced incidence of leaf mould compared to the untreated on 19 June (2 weeks after inoculation), Amistar treated plots contained fewest infected plants (Table 2). At the earlier 11 June assessment, Amistar, Switch and Teldor had significantly reduced disease severity, as had the biological HDC F186, whereas at the 19 June assessment, no treatments resulted in a severity significantly different to the untreated. Amistar, Signum and Teldor did show a trend for a reduction in severity at this assessment. The biological products Serenade ASO and HDC F185 also showed a trend towards a reduction in disease severity at the later assessment (Table 2). In terms of spray timing, the effect of spray timing was found to be significant. On 11 June there was significantly more disease (% leaf area affected) in the plots sprayed at +5 days than in plots sprayed at -1 or 0 days. There was also an observable trend for the efficacy of most products to decrease when used after inoculation at both assessment dates (Table 2). Therefore, we can conclude that the tested products are most effective when applied as protectants.

Amistar and Prestop were seen to cause phytotoxicity when applied at the time plants were bagged to create conditions conducive to infection (Fig 1), causing yellowing and leaf distortion of the younger leaves at the head of the plant. Amistar is known to cause such a reaction in warm temperatures and high humidity, but this reaction to Prestop has not been observed before. Neither Amistar nor Prestop caused phytotoxicity when the treatment was applied before or after bagging. No differences in crop vigour that were not attributable to phytotoxicity were observed during the trial.

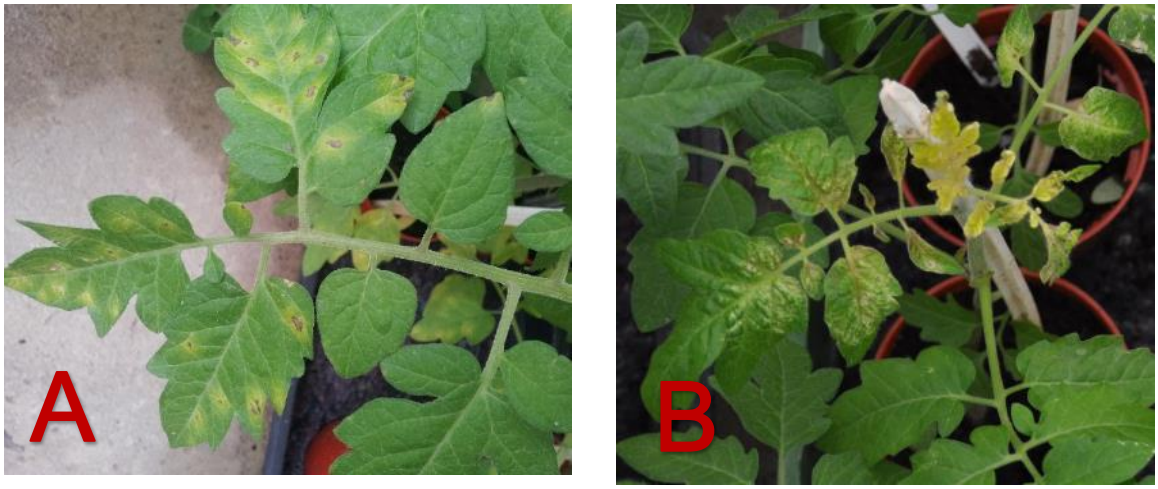

Figure 2. A – Symptoms of leaf mould 2 weeks after inoculation. B – Phytotoxicity following application of Amistar and imposed high humidity.

Products were further evaluated in another experiment on cv. Gardener's Delight. Fungicides were applied once at 3 days before inoculation or 3 days after inoculation; biofungicides were applied twice at -7 and 0 days before inoculation (Table 3).

Table 3. The effect of the best performing plant protection products on tomato leaf mould at 20 days after inoculation (Experiment 3) – 2014

Treatment	Application timing (days relative to inoculation)				% leaves affected	% leaf area affected	% area of inoculated layer affected
	-7	-3	0	3	24 July	31 July	31 July
1. Untreated					30	26.0	55.6
2. Serenade ASO	✓		✓		30	20.0	**31.2**
3. HDC F185	✓		✓		30	20.0	**28.7**
4. HDC F186	✓		✓		30	17.5	43.8
5. Amistar		✓			**10**	**8.5**	**6.9**
6. Switch		✓			**0**	**2.9**	**2.1**
7. Teldor		✓			30	23.8	37.5
8. Amistar				✓	**10**	**2.8**	**3.8**
9. Switch				✓	**10**	**0.5**	**0.4**
10. Teldor				✓	30	**10.5**	**17.5**
P value					<0.001	<0.001	<0.001
LSD between treatments					0.14	10.17	22.98
LSD vs. untreated					0.12	8.80	19.90

Values in bold are significantly different from untreated plants.

This experiment can be viewed as a more difficult test of the plant protection products than previously, as the severity achieved in untreated plots reached 26% leaf area affected. Perhaps because of this increased disease pressure, the biological products tested did not perform well against *P. fulva*. Of the three biological fungicides tested, only Serenade ASO and HDC F185 resulted in a significant reduction in leaf mould at the final assessment, and only when the inoculated leaf layer was assessed alone. HDC F186 did not give any observable reduction in leaf mould at any of the assessments. Of the conventional fungicide products, both Switch and Amistar significantly reduced disease incidence and severity at every assessment when applied at 3 days before inoculation (Table 3).

It should be noted that products were applied only once (Experiment 2), or once for fungicides and twice for biofungicides (Experiment 3) in line with experiment objectives. All of the registered products can be applied several times in a commercial setting (see Table 5) and it is possible that greater levels of control may be achieved where multiple sprays are used.

Objective 3 – Efficacy of disinfectants for reduction of *P. fulva*

Six commercially available disinfectant products were tested for efficacy against *P. fulva* in a series of laboratory experiments.

Initially, using specialised agar plates, disinfectants were tested against the spores and the mycelium of *P. fulva. P. fulva* spores and mycelium were challenged with the disinfectant at their recommended and half rates, and for 5 or 30 minutes at each rate. All of the disinfectants tested were effective when used at their recommended rate with 30 minutes of exposure.

Products were generally more effective against spores than against mycelium, and the products were not statistically different from one another in their efficacy. Those that maintained good efficacy when used at half rate or for only 5 minutes against mycelium (Jet 5, Unifect G, Menno Florades and Hortisept Pro) were used in two further experiments. In a comparison of disinfectants on four surfaces (aluminium, concrete, glass and plastic) Unifect G appeared the most effective against total fungal and bacterial growth; and aluminium, glass and plastic appeared easier to disinfect than concrete. However, no results were obtained specifically for *P. fulva* as the fungus was not recovered, not even from the untreated controls. It is likely growth on agar plates was swamped by more rapidly growing bacteria and fungi recovered concomitantly from the test surfaces. In a final experiment, the best performing products were tested against infected crop debris, which was used to inoculate tomato plants cv. Gardener's Delight, to determine if the debris was still infective (Table 4).

Table 4. Effect of disinfectant treatment on infectivity of tomato leaf debris affected by *P. fulva* – ADAS Boxworth, 2014

Treatment	% leaf area affected		% area affected inoculated leaf layer	
	12 Sep	19 Sep	12 Sep	19 Sep
1. Untreated	3.8	13.0	14.3	26.3
2. Jet 5	**0.6**	**6.8**	**5.4**	**16.3**
3. Unifect-G	**0.2**	**2.0**	**0.7**	**4.3**
4. Menno Florades	**0.1**	**0.8**	**0.3**	**2.6**
5. Hortisept Pro	**0.1**	**0.4**	**0.2**	**2.0**
P value	<0.001	<0.001	0.001	<0.001
LSD	1.114	3.405	6.117	5.240

At the first and second assessments, all disinfectant treatments succeeded in reducing disease transmission by infected leaf debris when compared to inoculation with untreated leaf debris (Table 4). There were initially no statistical differences between disinfection products. At the final assessment, however, differences between treatments became clearer. When

Jet 5 was used to treat leaf debris, significantly higher disease levels were observed than when debris was treated with the other disinfectant products. There were no statistical differences in efficacy between Hortisept Pro, Menno Florades and Unifect-G.

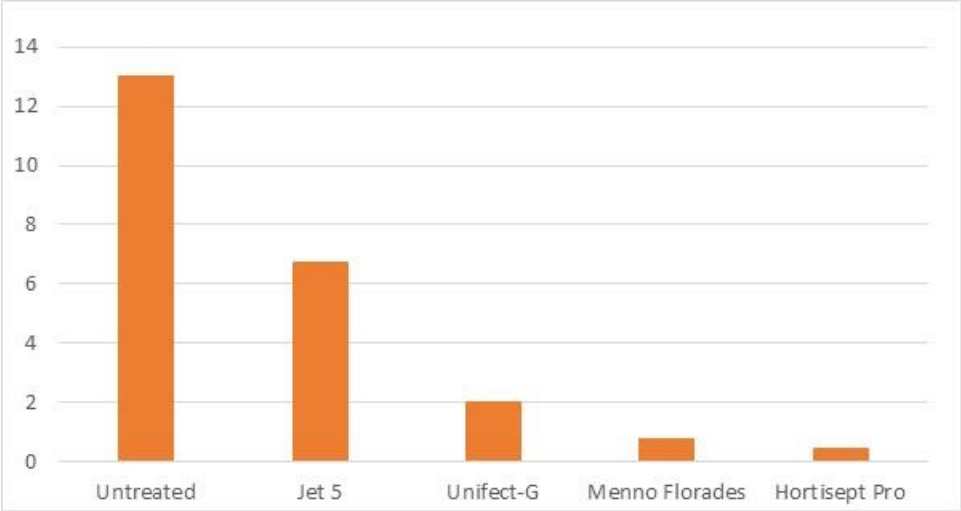

Figure 2. % leaf area affected by leaf mould after inoculation with disinfectant-treated leaf debris, 19 September 2014 – ADAS Boxworth

Financial Benefits

Good management of tomato leaf mould is likely to depend on both effective management of glasshouse humidity and use of effective plant protection products and disinfectants. Keeping relative humidity low in tomato glasshouses is already implemented as far as is allowed by other cropping factors. Similarly, large scale disinfection of glasshouses between crops is performed, but knowing which products will be most effective against *Passalora fulva* will allow this investment and labour to be more cost-effective. The disinfectant products found to be most effective in these trials are of comparable cost to others on the market. The plant protection products Amistar and Switch are already used in spray programmes against Botrytis, and a single spray application is estimated to cost £250 per hectare per season. There will also be the added benefit of providing some control of both diseases when sprays are applied, rather than having to add a new product to any spray programmes in place. However, spray timing may need to be adjusted to obtain the most effective control of leaf mould.

Action Points

- Minimise prolonged periods of high humidity (over 80% RH) as far as possible within the glasshouse
- Fungicides most effective against *P. fulva* include Amistar, Switch, and to a lesser extent, Teldor
- Although less effective than Amistar in this study, consider use of Signum when broad-spectrum disease control is required (eg Botrytis, leaf mould and powdery mildew)
- Use fungicides from two or more fungicide groups to reduce the risk of leaf mould developing resistance on your nursery; grower experience indicates that leaf mould can become resistant to azoxystrobin (Amistar) and it is likely that the related fungicide pyraclostrobin (in Signum) would also be ineffective in this situation.
- Biofungicides effective against *P. fulva* include Serenade ASO and a coded product (not currently registered for use on tomato)
- Generally, using plant protection products preventatively for control of leaf mould (i.e. at the very early stage in the disease epidemic) is more effective than using curatively
- The scientific literature indicates *P. fulva* can easily persist on a nursery between crops; carefully clean up all crop debris after crop removal, especially in cases where leaf mould has occurred
- After crop removal and clean-up, treat the glasshouse structure and floor with a suitable disinfectant so that any remaining very small leaf fragments are disinfected
- Disinfectants found most effective against *P. fulva* in this work include Hortisept Pro, Unifect-G and Menno Florades
- Regularly washing hands with soap and water or alcohol gel may help to prevent spread of leaf mould
- Disinfectant products are most effective when used at their full recommended rates, for as long a time as possible
- On more uneven, porous surfaces, such as concrete, it is likely that a disinfectant product will require a longer time to be fully effective

SCIENCE SECTION

Introduction

Tomato leaf mould (caused by *Passalora fulva*, previously known as *Cladosporium fulvum*) is often seen as a disease of the past, having primarily been a problem on unheated or part-heated tomatoes grown under protection. There are many strains of *Passalora fulva* and many corresponding resistance genes. For many years leaf mould largely ceased to be a problem commercially due to the incorporation of the *Cf-9* resistance gene into most commercial varieties. However, strains which can overcome the *Cf-9* and other resistance genes are known, and the occurrence of leaf mould is now increasing in the UK and elsewhere. Since 2000 there have been outbreaks of tomato leaf mould in the UK most years, affecting a range of varieties. For commercial reasons, little information is given by breeders on which leaf mould resistance genes are contained within their varieties.

Although there is no detailed knowledge of the relationship between % leaf area affected and yield loss, it is likely that a severe attack will reduce yield due to loss of photosynthetic area. An additional hazard with this disease is that some workers develop an allergic form of asthma when exposed to high concentrations of spores (Cobe, 1932; Hyde *et al*., 1956). Efforts to identify specific plant protection products with a high level of efficacy should be prioritised.

Passalora fulva produces only one spore form. These asexual spores (conidia) are produced in huge numbers and are spread by air currents, insects and on hands and clothing. They are reported to be very resistant to dryness and are believed to be the prime method by which the fungus persists from one crop to the next. Little information is available on the effectiveness of disinfection treatments in reducing inoculum of the pathogen at crop turn-around.

Information on tomato leaf mould has recently been reviewed and summarised in a detailed AHDB Horticulture Factsheet, 09/13. This project aimed to identify the most effective plant protection products and disinfectants to use as tools in integrated management of the disease in conventional and organic crops. The majority of products tested are already approved for use on protected tomato crops, though are not specifically used for control of tomato leaf mould. The regulatory status of products can be seen below (Table 5).

Table 5. Regulatory information for the products tested for control of tomato leaf mould

Product	a.i.	Rate (max conc.)	Max. no. sprays	Harvest interval (days)	MAPP	EAMU/Label	Final use
Amistar	azoxystrobin	0.1 L/100 L	4	3	10443	1685/01	30 June 2024
Signum	boscalid + pyraclostrobin	1.5 kg/ha	2	3	11450	0427/12	31 July 2019
Switch	cyprodinil + fludioxonil	1 kg/ha	3	3	15129	0302/11	31 Oct 2019
Teldor	fenhexamid	100 g /100 L	3	1	11229	2087/04	31 Dec 2015
Prestop	*Gliocladium catenulatum* strain J1446	0.5 kg /100 L	None stated	0	15103	On label	31 Jan 2020
Serenade ASO	*Bacillus subtilis* strain QST 713	10 L/ha	None stated	0	16139	0706/13	31 July 2019
HDC 185	Coded experimental product	-	-	-	-		-
HDC 186	Coded experimental product	-	-	-	-		-

Materials and methods

Experiment 1 – Evaluation of optimum infection conditions for *Passalora fulva* on young tomato plants

At the start of glasshouse experiments, ADAS had three confirmed isolates of *P. fulva*. Two of these isolates, BX14/13 and BX14/29 were obtained from tomato crops, and BX14/16 was a 1953 isolate obtained from CABI (*ex* tomato). A small scale pathogenicity test was run alongside Experiment 1 to determine the virulence of each of these isolates. Six plants per isolate were inoculated using a hand sprayer containing a 5 x 10^3 spores/ml spore suspension. Each plant received two sprays from below to each of the first two true leaves.

The main experiment on infection conditions was a factorial design with two factors (inoculum level and high humidity duration) at two and three levels respectively, plus an untreated control. Plot size was 4 plants, and the experiment contained 4 replicates. Results were examined by ANOVA and regression analysis as appropriate.

Tomato plants of cv. Gardener's Delight were grown in Levington M3 compost, in 10 cm diameter pots in a glasshouse at ADAS Boxworth. They were inoculated on 28th April 2014

at the 4-6 true leaf stage using a hand sprayer. Each plant was sprayed twice from below, to the point of run-off, to target the lower leaf surfaces of the first two true leaves, and plots were covered with polythene to enforce high humidity on plants for different periods (24, 48 and 96 hours). Plots were inoculated with either a high (1×10^5) or low (1×10^4) concentration of conidia per ml (Table 6). Control plots were sprayed with sterile distilled water. Temperature and humidity close to leaves was recorded using a USB logger placed in a central plot subjected to 96 hours of high humidity, and plants were grown in an unheated glasshouse at around 20°C (Appendix 1).

Table 6. List of treatments as applied to tomato cv. Gardener's Delight in Experiment 1 – ADAS Boxworth, 2014

Treatment	Inoculation	Humidity period (hours)
1.	Nil	24
2.	Nil	48
3.	Nil	96
4.	Low	24
5.	Low	48
6.	Low	96
7.	High	24
8.	High	48
9.	High	96

Plants were inspected for disease symptoms daily. Symptoms were assessed by recording incidence (number of plants affected in a plot, out of 4) and severity (% leaf area affected) for each plot. Crop vigour was assessed on a 0-5 scale where 0 equalled a dead plant, and 5 equalled a vigorous, healthy plant. Full assessments were done at 2 and 3 weeks after inoculation. Leaf samples were taken from the crop when first symptoms were seen and isolations onto PDA+S agar were carried out in the pathology laboratory at ADAS Boxworth to confirm that the symptoms observed were due to *P. fulva*.

Experiment 2 – Evaluation of efficacy of fungicide and biofungicide products against *Passalora fulva* on tomato

Plants of cv. Gardener's Delight were grown in Levington M3 compost, in 10 cm diameter pots in two adjacent glasshouse compartments at ADAS Boxworth. The experiment

contained four replicates (with four plants per plot), and plants were treated with plant protection products at one of five application timings. Products included four conventional fungicides and four biofungicides, applied at 5 and 1 days before inoculation, on the day of inoculation (before inoculation and allowed to dry), and 1 and 5 days after inoculation. As a number of spare plants were available, the experimental chemical HDC F188 was also tested on four plots, applied at 1 day after inoculation. Products were applied with medium spray quality at 100 L/ha water, using a backpack sprayer fitted with 04F110 nozzles. Details of products are shown in Table 7.

Table 7. Products applied to tomato cv. Gardener's Delight at five timings ranging from 5 days before to 5 days after inoculation with *P. fulva* – ADAS Boxworth, 2014 (Experiment 2)

Product	Active ingredient	Rate	EAMU/Label
1. Water control	-	-	
2. Amistar	Azoxystrobin	0.1 L / 100 L (1 ml /L)	1685/01
3. Signum	Boscalid + pyraclostrobin	1.5 kg/ha (1.5 g /ml)	0427/12
4. Switch	Cyprodinil + fludioxonil	1 kg/ha (1 g /ml)	0302/11
5. Teldor	Fenhexamid	0.1 kg/ha (0.1 g /L)	2087/04
6. Prestop	*Gliocladium catenulatum*	500 g / 100 L (5 g /L)	On label
7.Serenade ASO	*Bacillus subtilis*	10 L/ha (10 ml /L)	0706/13
8. HDC F185	–	0.5 g/L	Experimental approval
9. HDC F186	–	2.5 L/ha	Experimental approval

* The AEA held by ADAS is valid until 31 May 2017 and has reference number COP 2014/00736.

Using information from Experiment 1, plants were inoculated with the highest achievable rate of 2.8 x 10^4 spores/ml using a hand sprayer. Each plant was sprayed twice from below to target the lower leaf surfaces of the first two true leaves, and plots were covered with polythene for 96 hours following inoculation on the 28th May. This experiment was a factorial

design with two factors (product and application timings) at two and three levels respectively, plus an untreated control. Results were examined by ANOVA or regression analysis as appropriate.

Plants were inspected for disease symptoms daily. Symptoms were assessed by recording incidence (number of plants affected in a plot, out of four) and severity (% leaf area affected) for each plot. Phytotoxicity was assessed on a 0-9 scale, where 0 equalled no phytotoxicity and 9 equalled severe phytotoxicity. Crop vigour was assessed on a 0-5 scale where 0 equalled a dead plant, and 5 equalled a vigorous, healthy plant. Full assessments were done at 2 and 3 weeks after inoculation. At the final assessment, an assessment of % leaf area affected was also carried out only for the leaf layer to which inoculum was applied.

The experiment was watered daily directly to the capillary matting at the base of trays to avoid washing away spores or spreading them between plots. Two separate glasshouses had to be used to contain all the treatments included in the experiments, but each compartment was controlled by the same system so that fan settings etc. were the same in both. Temperature and humidity data can be seen in Appendix 1.

Experiment 3 – Further evaluation of the efficacy of the best performing fungicide and biofungicide treatments

After Experiment 2 the best products and timings were carried forward for further testing (Table 8). Products were applied with medium spray quality at 100 L/ha water, using a backpack sprayer fitted with 04F110 nozzles. The products applied on day 0 were applied and allowed to dry before inoculation.

Table 8. Products and application timings as applied to tomato cv. Gardener's Delight – ADAS Boxworth, 2014 (Experiment 3)

Treatment no.	Product	Timing	Comment
1.	Untreated	0	
2.	Serenade ASO	-7, 0	2 applications of best 3 biofungicides as protectants
3.	HDC F185	-7, 0	
4.	HDC F186	-7, 0	
5.	Amistar	-3	1 spray of best 3 fungicides, as protectants
6.	Switch	-3	
7.	Teldor	-3	
8.	Amistar	+3	1 spray of best 3 fungicides, as curatives
9.	Switch	+3	
10.	Teldor	+3	
11.	Untreated	0	

Plants of cv. Gardener's Delight were grown in Levington M3 compost, in 10 cm in diameter pots in a glasshouse at ADAS Boxworth. Plants were inoculated with a spore suspension of 2.9×10^4 spores/ml on 11th July (as this was the highest achievable spore concentration with the cultures available). Each plant was sprayed twice from below to target the lower leaf surfaces of the first two true leaves, and plots were covered with polythene for 96 hours.

The experiment was watered daily directly to the capillary matting at the base of trays to avoid washing away spores or spreading them between plots. Two separate glasshouses had to be used to contain all the treatments included in the experiments, but each compartment was controlled by the same system so that fan settings etc. were the same in both. Temperature and humidity data can be seen in Appendix 1.

This experiment was a factorial design with two factors (product and application timings) at two and three levels respectively, plus an untreated control. The experiment was replicated four times, and there were 4 plants to a plot. Results were examined by ANOVA or regression analysis as appropriate.

Plants were assessed as described in Experiment 2.

Experiment 4 – Testing of disinfectants against spores of *P. fulva*

Testing commercially available disinfectant products against spores of *P. fulva* was carried out using methods as used in AHDB Horticulture Project PE 001a in the pathology laboratory at ADAS Boxworth, on the 2nd July 2014. A spore solution of *P. fulva* of $1x10^5$ spores/ml in sterile distilled water was made up and the spores exposed to each disinfectant product at their full and half recommended rates for either 5 or 30 minutes. The products tested are shown below (Table 9).

Table 9. Products tested against spores of *Passalora fulva* – ADAS Boxworth, 2014 (Experiment 4)

Treatment	Product	Active ingredient	Recommended rate
1.	Untreated	-	-
2.	Bleach	5% Sodium hypochlorite	1:10
3.	Fam 30	Iodophor	1:90
4.	Hortisept Pro	Quaternary ammonium compound + inorganic acids	1:25
5.	Jet 5	Hydrogen peroxide + peroxyacetic acid	1:125
6.	Menno Florades	Benzoic acid	1:25
7.	Unifect G	Glutaraldehyde + QAC	1:50

In Universal tubes spores were exposed to products at the appropriate rate and timing, made up to 10 ml with sterile distilled water. After 5 or 30 minutes, tubes were centrifuged at 2000 rpm for 5 minutes, so that a pellet of spores formed. The disinfectant liquid was pipetted off and removed, and the pellet re-suspended in 5 ml of sterile distilled water. This was performed three times to ensure the spores were fully rinsed of disinfectant. The final 5 ml spore suspension for each rate x timing combination was then plated out onto PDA+S agar.

Using a specialised 5 x 5 cm multi-cell agar plate, 20 ul of each treated spore solution was pipetted, using sterilised pipette tips, into the appropriate cell. Two plates were set up for each disinfectant product, giving a total of 10 replicates. There were 10 replicates of untreated spores, which were also centrifuged and rinsed 3 times. Plates were incubated at 22°C on a 12 hour light/dark cycle.

Each timing and rate combination per treatment was assessed out of 10 after 7 days, giving a % fungal growth. No growth was assessed as 0, whereas visible fungal growth was assessed as 1. Photographs of a representative plate for each treatment were also taken. No statistical analysis was carried out as this was deemed sufficient.

In addition to the main spore experiment, the effects of common hand disinfection practices as used in nurseries on spores was also tested. Spores of *P. fulva* from PDA+S agar plates were transferred onto hands and then dotted with a finger around an agar plate. This was repeated after washing hands with soap and water, and after using alcohol gel. Resulting growth was photographed.

Experiment 5 – Testing of disinfectants against mycelium of *P. fulva*

On 31st July, the disinfectant products tested in Experiment 4 (Table 8) were tested against mycelium of *P. fulva* in the ADAS Pathology laboratory. *P. fulva* was grown on PDA+S agar plates covered with sterile filter paper discs, 0.5 cm in diameter. The filter paper discs were then picked off once covered in fungal growth (after a month of growth), and were exposed to each disinfectant product at their full and half recommended rates for either 5 or 30 minutes. Discs were placed in Universal tubes filled with 10 ml of disinfectant of differing rates and for differing periods of time. Once the set time period had elapsed the discs were removed from the disinfectant product and rinsed three times in sterile distilled water. Discs were then left to dry in a laminar flow cabinet for approximately 30 minutes.

Using a specialised 5 x 5 cm multi-cell PDA+S agar plate, each disc was placed into the appropriate cell with sterilised tweezers. One plate was set up for each disinfectant product, giving a total of five replicates. There were five replicates of untreated mycelial discs on each plate, which were immersed in sterile distilled water and rinsed three times, then dried. Plates were incubated at 22°C on a 12 hour light/dark cycle.

Each timing and rate combination per treatment was assessed out of five after 7 days, giving a % fungal growth. No growth was assessed as 0, whereas visible fungal growth was assessed as 1. Photographs of a representative plate for each treatment were also taken. No statistical analysis was carried out as this was deemed sufficient.

Experiment 6 – Testing of disinfectants on a variety of surfaces commonly found on nurseries against spores of *P. fulva*

On August 5th, a variety of surfaces commonly found on nurseries were sourced, and 15 cm^2 squares marked out on them using electrical tape. A pane of glass, a concrete slab, a sheet of aluminium and rigid plastic trays were washed with soap and water and allowed to dry, taken into the ADAS Pathology laboratory, and then cleaned with 100% ethanol and allowed to dry. A spore suspension of *P. fulva* at $1x10^5$ spores per ml was applied to each marked square using a hand sprayer, and allowed to dry. Two sprays were applied to each square to give good coverage, equating to approximately 2 ml per marked out square. A square on each of the four surfaces was treated with the disinfectant products detailed in Table 8 at their

recommended rates, also using hand sprayers (c. 10 ml/m^2). The untreated surfaces were sprayed with sterile distilled water. Once the treatments applied to the different surfaces had dried, ten swabs were taken from each square using new, clean cotton buds. Squares were swabbed in a cross-hatched pattern in a zig-zagging motion, over a length of approximately 20 cm to ensure all areas of the treated square were sampled. Each swab was used to streak across approximately 10 cm on a PDA+S agar plate. Two swabs were streaked on a single plate, giving five agar plates per surface x treatment combination. Plates were incubated at 22°C on a 12 hour light/dark cycle and assessed for growth 7 days later.

Experiment 7 – Testing of disinfectants on *P. fulva* infected leaf debris by subsequent transmission testing on glasshouse tomato

Plants of cv. Gardener's Delight were grown in Levington M3 compost, in 10 cm diameter pots in a glasshouse at ADAS Boxworth. Plants were inoculated using leaves heavily infected with *P. fulva*, sourced from a commercial glasshouse, and subsequently treated with one of five disinfectant products. The control treatment was inoculation with infected leaves sprayed with sterile distilled water to point of run-off. For each disinfectant treatment, the test product was used at its full recommended rate and applied to the leaf debris using a hand sprayer until point of run off. Once the debris had dried, the trial was inoculated with a set weight of leaf debris, 0.75 g per plot. The debris was pressed against the lower surface of the two lowest true leaves on each plant, and any remaining debris sprinkled over the plot. Once inoculated, plots were covered with polythene for 96 hours. Each plot was inoculated and then covered before another plot was inoculated, to avoid spread of leaf debris from one plot to another.

The products carried forward for testing against infected leaf debris were Jet 5, Menno Florades, Hortisept Pro and Unifect-G. This was due to their efficacy in the previous three experiments, and their level of use by growers. Though performing well, bleach was not carried forward as it is not used as often as the other similarly performing products.

This experiment was a blocked design with four treatments, plus an untreated control, replicated four times with 4 plants per plot. Results were examined by ANOVA or regression analysis as appropriate.

The experiment was watered daily directly to the capillary matting at the base of trays to avoid washing away spores or spreading them between plots. Temperature and humidity data can be seen in Appendix 1.

Plants were assessed as described previously.

Results

Experiment 1 – Evaluation of optimum infection conditions of *Passalora fulva* on tomato

When each of the isolates held by ADAS were tested on tomato, disease developed at similar levels with all isolates. Despite appearing quite different in culture, the oldest isolate, BX14/16, provided by CABI, produced the same symptoms on tomato as the two current isolates, BX14/13 and BX14/29. As a result of this preliminary test, BX14/13 was selected for use in the main trials as it was the fastest growing of the two newer isolates on agar (data not presented).

In the main experiment, first symptoms of leaf mould were seen on 9th May, 11 days after inoculation, visible as faint yellow spots on the upper leaf surface. The disease increased in severity greatly between 2 and 3 weeks after inoculation, progressing to cover almost entire leaflets in some plots. A significant interaction effect indicated that disease development is greatest in the case of high inoculum concentration, and an extended period of high humidity ($p < 0.001$). Disease severity was significantly higher in plots inoculated with 1×10^5 conidia per ml and covered with polythene for 96 hours after inoculation ($p = 0.034$) (Table 10).

Effects on crop vigour were in line with symptom severity, with plants subjected to high inoculum levels and high humidity periods generally having the lowest vigour. However, it must also be noted that humidity alone also had a significant effect on vigour ($p = 0.034$), indicating that high humidity could have stunted and/or damaged plants independent of disease (Table 11).

Table 10. Effect of inoculation rate and humidity period on development of *P. fulva* – ADAS Boxworth, 2014 (Experiment 1)

Treatment	Inoculation rate	Humidity period (h)	Severity (% leaf area affected)		Crop vigour (0-5 index)
			12 May	15 May	15 May
1	Uninoculated	24	0.1	0.2	4.8
2	Uninoculated	48	0.0	0.0	5.0
3	Uninoculated	96	0.0	0.0	5.0
4	Low rate	24	0.3	5.0	5.0
5	Low rate	48	1.0	4.5	4.8
6	Low rate	96	1.8	**13.8**	5.0
7	High rate	24	1.8	8.8	5.0
8	High rate	48	3.3	**15.0**	5.0
9	High rate	96	**15.0**	**35.0**	**3.5**
Probability (F value) Inoculation x humidity			<0.001	0.005	<0.001
LSD (24 d.f.)			3.55	9.31	0.62

*Values in bold are significantly different from uninoculated plants.

Table 11. Table of means showing effects of inoculation rate and humidity period on disease development – ADAS Boxworth, 2014 (Experiment 1)

Factor	Mean severity (% leaf area affected)		Crop vigour (0-5 index)
	12 May	15 May	15 May
<u>Inoculation rate</u>			
Nil	0	0.1	4.9
Low	1.0	7.8	4.9
High	6.7	19.6	4.5
<u>Humidity period</u>			
24	0.7	4.7	4.9
48	1.4	6.5	4.9
96	5.6	16.3	4.5

Based on these results it was decided that inoculation in subsequent glasshouse trials would use the highest spore concentration achievable and that plots would be covered for 96 hours post-inoculation to achieve adequate levels of disease against which products would be tested.

Experiment 2 – Evaluation of efficacy of fungicide and biofungicide products against *Passalora fulva* on tomato

First disease symptoms were noted on the 9th June, 12 days after inoculation, and symptoms continued to increase in incidence and severity over the course of the experiment. Results averaged across spray timings show that all treatments reduced leaf mould incidence compared to the untreated on the 19th June (Table 12); there were no significant differences between product treatments at this assessment date. Amistar, Switch, Teldor and HDC F186 resulted in disease severities significantly lower than the untreated, at the earlier assessment (11 June). Results averaged across all products show that the 'curative' treatment timing, applied 5 days after inoculation, was less effective than spray application at the time of inoculation.

Table 12. Effect of plant protection product and spray timing on tomato leaf mould – ADAS Boxworth, 2014 (Experiment 2)

Factor	Mean % plants affected		Mean % leaf area affected (severity)	
	11 June	19 June	11 June	19 June
Product				
1. Water control	93.8 (8.4)	100 (<0.1)	1.8	4.8
2. Amistar	24.7 (6.0)	**47.3** (6.4)	**0.2**	0.9
3. Signum	46.8 (6.0)	**65.4** (5.0)	1.0	2.4
4. Switch	40.1 (7.1)	**70.4** (5.5)	**0.7**	2.7
5. Teldor	46.8 (6.9)	**73.8** (5.9)	**0.7**	1.5
6. Prestop	58.4 (6.7)	**86.4** (4.6)	1.2	2.6
7. Serenade ASO	42.7 (5.1)	**56.3** (3.8)	0.9	2.3
8. HDC F185	46.3 (6.4)	**76.1** (5.5)	0.9	1.9
9. HDC F186	42.8 (6.8)	**72.6** (5.8)	**0.7**	2.5
P value (120 d.f.)	0.143	0.002	0.033	0.142
LSD	-	-	0.9425	2.356
Timing				
-5	40.6 (5.2)	67.2 (4.0)	0.6	1.7
-1	32.9 (4.8)	58.6 (4.9)	0.4	1.3
0	36.8 (5.1)	65.6 (4.2)	0.4	2.1
1	50.0 (5.3)	72.7 (4.2)	1.0	2.0
5	58.6 (4.9)	79.0 (3.8)	**1.4**	3.4
P value (120 d.f.)	0.016	0.051	<0.001	0.005
LSD	-	-	0.9126	2.281

*values in bold are significantly different from the water control (upper set of columns) or the day 0 timing (lower set of columns); ( ) – standard error.

The interaction between product and application timing was highly significant ($p<0.001$), meaning that the best product differs with time of application (Table 13). Amistar was the only product that significantly reduced disease severity on whole plants irrespective of spray timing. Most conventional fungicides were also able to significantly control *P. fulva* when applied at 1 day after inoculation, potentially due to a high level of contact achieved at a time fungal spores are germinating and vulnerable. The control offered by Signum was significant when applied at earlier timings (-1 days), but at both assessments levels of disease on plots treated with Signum at 5 days after inoculation had more disease present than on untreated plots. At 2 weeks following inoculation, Switch provided significant disease control when applied after inoculation, though this did not persist to 3 weeks after inoculation. Teldor was not found to be effective when applied at 5 days after inoculation at either assessment, but generally gave good control when applied earlier.

Of the biologicals, Serenade ASO was most effective, and had good efficacy when applied before and on the day of inoculation. The control offered by Prestop did not seem to be so influenced by application timing, but significant control was achieved at both assessments when applied 5 days before inoculation. HDC F185 and HDC F186 gave significant control at both assessments when they were applied before inoculation, and there was a noticeable trend for efficacy of all the biologicals tested to decline when applied after inoculation. HDC F185 was the exception to this when applied at 5 days after inoculation, which gave significant control. It is possible this biocontrol agent needed sufficient time to build up a sufficient population. Position in the glasshouses (arranged in blocks 1-4) also seemed to have an effect on severity of leaf mould, potentially linked to humidity 'hot spots' ($p <0.001$ on 11th June).

Table 13. Effect of plant protection product and spray timing on tomato leaf mould, % leaf area affected across the whole plant, 11th and 19th June (Experiment 2) – ADAS Boxworth, 2014

Product	Spray timing (days relative to inoculation)				
	-5	-1	0	1	5
Two weeks after inoculation					
1. Untreated	-	-	1.8	-	-
2. Amistar	**0.1**	**0.2**	**0.1**	**0.5**	**0.2**
3. Signum	1.5	**0**	1.1	0.8	2.1
4. Switch	1.7	**0.3**	0.7	**0.2**	**0.4**
5. Teldor	0.8	**0.3**	**0.1**	**0.5**	1.8
6. Prestop	**0.3**	1.9	**0.5**	0.8	2.5
7. Serenade ASO	**<0.1**	0.7	**0**	1.4	2.4
8. HDC F185	**0.5**	**0.2**	0.6	2.8	**0.1**
9. HDC F186	**0.3**	**0.1**	**0.3**	1.3	1.5
P value			<0.001		
LSD (120 d.f.)			1.2167		
Three weeks after inoculation					
1. Untreated	-	-	4.8	-	-
2. Amistar	**0.2**	**0.9**	**1.3**	**1.4**	**0.9**
3. Signum	2.5	**0.2**	3.0	**0.4**	5.8
4. Switch	5.4	**1.7**	2.9	**0.2**	3.3
5. Teldor	**1.2**	**0.9**	**1.1**	**1.3**	3.3
6. Prestop	**1.3**	3.5	2.5	**1.5**	4.3
7. Serenade ASO	**0.1**	1.8	**<0.1**	4	5.8
8. HDC F185	**1.5**	**1.4**	2.0	3.8	**1.0**
9. HDC F186	**1.4**	**0.5**	4.1	4	2.6
P value			0.004		
LSD (120 d.f.)			3.041		

*Figures in bold are significantly different from the untreated.

When the inoculated leaf layer was assessed alone, the only products to give significant control were Amistar when applied at -5 days, and Signum when applied at -1 day (Table 14).

Table 14. Effect of plant protection product and spray timing on tomato leaf mould, % leaf area affected of the leaf layer inoculated, 23rd June – ADAS Boxworth, 2014 (Experiment 2)

Product	Spray timing (days relative to inoculation)				
	-5	-1	0	1	5
Three weeks after inoculation					
1. Untreated	-	-	22.0	-	-
2. Amistar	**6.3**	11.3	10.0	18.8	17.5
3. Signum	27.5	**5.0**	17.0	9.8	20.0
4. Switch	27.5	11.3	16.3	11.0	30.8
5. Teldor	15.0	10.8	9.5	13.8	20.8
6. Prestop	20.0	17.5	18.3	16.3	35.0
7. Serenade ASO	10.0	17.5	12.5	20.0	22.5
8. HDC F185	16.3	12.5	10.0	26.3	10.0
9. HDC F186	18.3	12.2	14.0	25.0	17.0
P value			0.163		
LSD (120 d.f.)			14.731		

*Figures in bold are significantly different from the untreated.

Table 15 illustrates the differences in disease incidence observed in the trial at the second assessment date. In terms of reducing disease incidence, all conventional products had significant efficacy when applied at 1 day before inoculation. When applied at 5 days before inoculation, or on the day of inoculation, only Amistar significantly reduced disease incidence. When applied after inoculation, both Signum (when applied at 1 day after), Amistar (when applied at 5 days after) and Switch (when applied at either 1 or 5 days after) reduced disease incidence significantly. Of the biological products, Serenade ASO had highest efficacy, reducing incidence to just 6.2% of plants when applied on the day of inoculation. Serenade ASO also significantly reduced incidence when used preventatively at 5 or 1 day(s) before inoculation. However, when applied after inoculation, all plants treated with Serenade ASO became infected. Prestop did not significantly reduce disease incidence at any application timing. HDC F185 was effective when applied on the day of inoculation or at 5 days after inoculation, whereas HDC F186 was only successful in reducing incidence significantly when applied at 1 day before.

Table 15. Effect of plant protection product and spray timing on tomato leaf mould, % plants affected, 19th June (Experiment 2) – ADAS Boxworth, 2014

Product	Spray timing (days relative to inoculation)				
	-5	-1	0	1	5
1. Untreated	-	-	100 (0.0)	-	-
2. Amistar	**25.0** (13.3)	**56.3** (15.1)	**37.5** (14.7)	75.0 (13.3)	**43.8** (15.1)
3. Signum	87.5 (10.3)	**18.8** (12.0)	81.3 (12.0)	**37.5** (14.7)	100 (<0.01)
4. Switch	100 (<0.01)	**56.3** (15.1)	87.5 (10.3)	**43.8** (15.1)	**62.5** (14.8)
5. Teldor	75.0 (13.3)	**56.3** (15.1)	75.0 (13.3)	75.0 (13.3)	87.5 (10.3)
6. Prestop	81.3 (12.0)	87.5 (10.3)	93.8 (7.6)	75.0 (13.3)	93.8 (7.6)
7. Serenade ASO	**12.5** (10.3)	**68.8** (14.2)	**6.2** (7.6)	100 (<0.01)	100 (<0.01)
8. HDC F185	75.0 (13.3)	81.3 (12.0)	**68.8** (14.2)	100 (<0.01)	**56.3** (15.1)
9. HDC F186	81.3 (12.0)	**43.8** (15.1)	75.0 (13.3)	75.0 (13.3)	87.5 (10.3)
P value			<0.001		

Standard errors in parentheses; figures in bold show product x timing treatments that significantly reduced disease compared with untreated plants (100% affected).

Amistar and Prestop were seen to cause phytotoxicity, causing yellowing and leaf distortion of the younger leaves at the head of the plant. Amistar is known to cause such a reaction in warm temperatures (as detailed in EAMU 1533/02, which states Amistar should not be applied to stressed plants after transplanting, on young plants under low light or poor drying conditions, or at temperatures above 30°C or below 10 °C), but this reaction to Prestop has not been observed before. This reaction occurred only in plots treated at 1 day after inoculation, with Amistar treated plots having an average phytotoxicity index of 4.5 out of 9, and Prestop treated plots having a score of 2 out of 9. No differences in crop vigour that were not attributable to phytotoxicity were observed during the trial.

An additional experiment was also run alongside the main trial. Using some spare plants an experimental product, HDC F188, approved for use on tomatoes overseas but not currently approved for use in the UK, was tested against *P. fulva* 1 day after inoculation (Table 16). When compared only to the other experimental treatments at this timing HDC F188 was not seen to be significantly different from the other products.

Table 16. The efficacy of HDC F188 compared with main experimental treatments when sprayed at 1 day after inoculation – 2014 (Experiment 2)

Treatment	Mean % plants affected		% leaf area affected (severity)		% inoculated layer
	11 June	19 June	11 June	19 June	23 June
2. Amistar	50.0 (16.5)	75.0 (11.1)	0.53	1.4	18.8
3. Signum	18.8 (13.0)	37.5 (12.4)	0.08	0.4	9.8
4. Switch	25.0 (14.3)	43.8 (12.7)	0.18	0.2	11.0
5. Teldor	43.8 (16.3)	75.0(11.2)	0.50	1.3	13.8
6. Prestop	43.8 (16.3)	75.0 (11.2)	0.75	1.5	16.2
7. Serenade ASO	75.0 (14.7)	100.0 (<0.1)	1.38	4.0	20.0
8. HDC F185	93.8 (8.4)	100.0 (<0.1)	2.88	3.8	26.2
9. HDC F186	50.0 (16.5)	75.0 (11.2)	1.28	4.0	25.0
10. HDC F188	75.0 (14.7)	81.3 (10.1)	1.88	2.9	23.8
P value	0.051	0.003	0.006	0.083	0.11
LSD (24 d.f.)	-	-	1.374	3.105	12.86

Standard errors in parentheses

Experiment 3 – Further evaluation of the efficacy of the best performing fungicide and biofungicide treatments against tomato leaf mould

First disease symptoms were noted on the 24th July, 13 days after inoculation, and symptoms increased over the course of the experiment to give the highest disease severity in the untreated observed throughout the project. As a result, this experiment can be viewed as a more difficult test of the plant protection products than Experiment 2. Perhaps because of this increased disease pressure, the biological products tested did not perform well against *P. fulva* in Experiment 3. Of the three biological fungicides tested, Serenade ASO and HDC F185 only resulted in a significant reduction in leaf mould at the final assessment, and only when the inoculated leaf layer was assessed alone. HDC F186 did not give any observable reduction in leaf mould at any of the assessments.

Of the conventional fungicide products, both Switch and Amistar significantly reduced disease incidence and severity at every assessment when applied at 3 days before inoculation (Table 17). Teldor did not significantly reduce disease at any assessment timing compared to the untreated plots when applied 3 days before inoculation. When applied 3 days after inoculation, Switch still gave significant control at all assessments. Amistar also reduced disease incidence significantly 2 weeks after inoculation, and had significantly reduced disease severity compared to untreated plots 3 weeks after inoculation. When applied at 3 days after inoculation, Teldor treated plots had a significant reduction in disease compared to untreated plots. For Switch and Amistar, the disease levels achieved after spraying either 3 days before or 3 days after inoculation were not significantly different from one another. In

the case of Teldor, however, it appeared that spraying at +3 days was significantly better than spraying at -3 days.

Table 17. Effect of most promising plant protection products on incidence and severity of *P. fulva* at 2 and 3 weeks after inoculation– 2014 (Experiment 3)

Treatment	Application timing (days relative to inoculation)				% leaves affected	% leaf area affected		% inoculated layer affected	
	-7	-3	0	3	24 July	24 July	31 July	24 July	31 July
1. Untreated			✓		29	2.1	26.0	6.7	55.6
2. Serenade ASO	✓		✓		28	2.3	20.0	7.0	**31.2**
3. HDC F185	✓		✓		29	1.9	20.0	5.6	**28.7**
4. HDC F186	✓		✓		31	2.0	17.5	6.3	43.8
5. Amistar		✓			**5**	**0.1**	**8.5**	**0.3**	**6.9**
6. Switch		✓			**2**	**0.1**	**2.9**	**0.3**	**2.1**
7. Teldor		✓			34	2.2	23.8	7.0	37.5
8. Amistar				✓	**9**	0.8	**2.8**	3.5	**3.8**
9. Switch				✓	**6**	**0.1**	**0.5**	**0.3**	**0.4**
10. Teldor				✓	25	1.0	**10.5**	3.2	**17.5**
P value					<0.001	0.041	<0.001	0.011	<0.001
LSD vs. treatment					0.14	1.88	10.17	5.05	22.98
LSD vs. untreated					0.12	1.63	8.80	4.38	19.90

At the assessment on 24th June, none of the biological products tested significantly reduced levels of *P. fulva*. At the assessment three weeks after inoculation on the 31st July Serenade ASO and HDC F185 had reduced the severity of disease on the leaf layer that was inoculated, but not across the plant as a whole.

No differences in crop vigour were observed in the trial, and very mild phytotoxicity was observed in only one plot treated with Amistar at -3 days.

Experiment 4 – Testing of disinfectants against spores of *P. fulva*

All disinfection products tested prevented growth of P. fulva spores when used at their recommended rate for 30 minutes. All products reduced the viability of *P. fulva* spores when plated onto agar, at both half and recommended rates and when exposed for 5 or 30 minutes (Table 18). As expected, exposure for 30 minutes was more effective in killing spores than exposure for 5 minutes. Additionally, in this test limited differentiation between half and full rates was seen, although small numbers of spores compared to the untreated were noted to survive when half the rate was used for 5 minutes for the treatments Hortisept Pro, Jet 5, Menno Florades and Unifect-G. A small amount of growth was also observed for Jet 5 when used at the recommended rate for 5 minutes, but 30 minutes of either the recommended or half rate was sufficient for total spore death. Examples of the test plates are shown in Figure 3.

Table 18. % fungal growth observed after treatment with six disinfectant products for 5 or 30 minutes, at full or half recommended rates – ADAS Boxworth, 2014 (Experiment 4)

Treatment		Rate (recommended)	Timing (minutes)	% fungal growth (out of 10 wells)
2	Bleach	0	0	100
		Half	5	0
		Half	30	0
		Full	5	0
		Full	30	0
3	FAM 30	0	0	100
		Half	5	0
		Half	30	0
		Full	5	0
		Full	30	0
4	Hortisept Pro	0	0	100
		Half	5	40
		Half	30	0
		Full	5	0
		Full	30	0
5	JET 5	0	0	100
		Half	5	20
		Half	30	0
		Full	5	10
		Full	30	0
6	MENNO Florades	0	0	100
		Half	5	20
		Half	30	0
		Full	5	0
		Full	30	0
7	Unifect-G	0	0	100
		Half	5	20
		Half	30	0
		Full	5	0
		Full	30	0

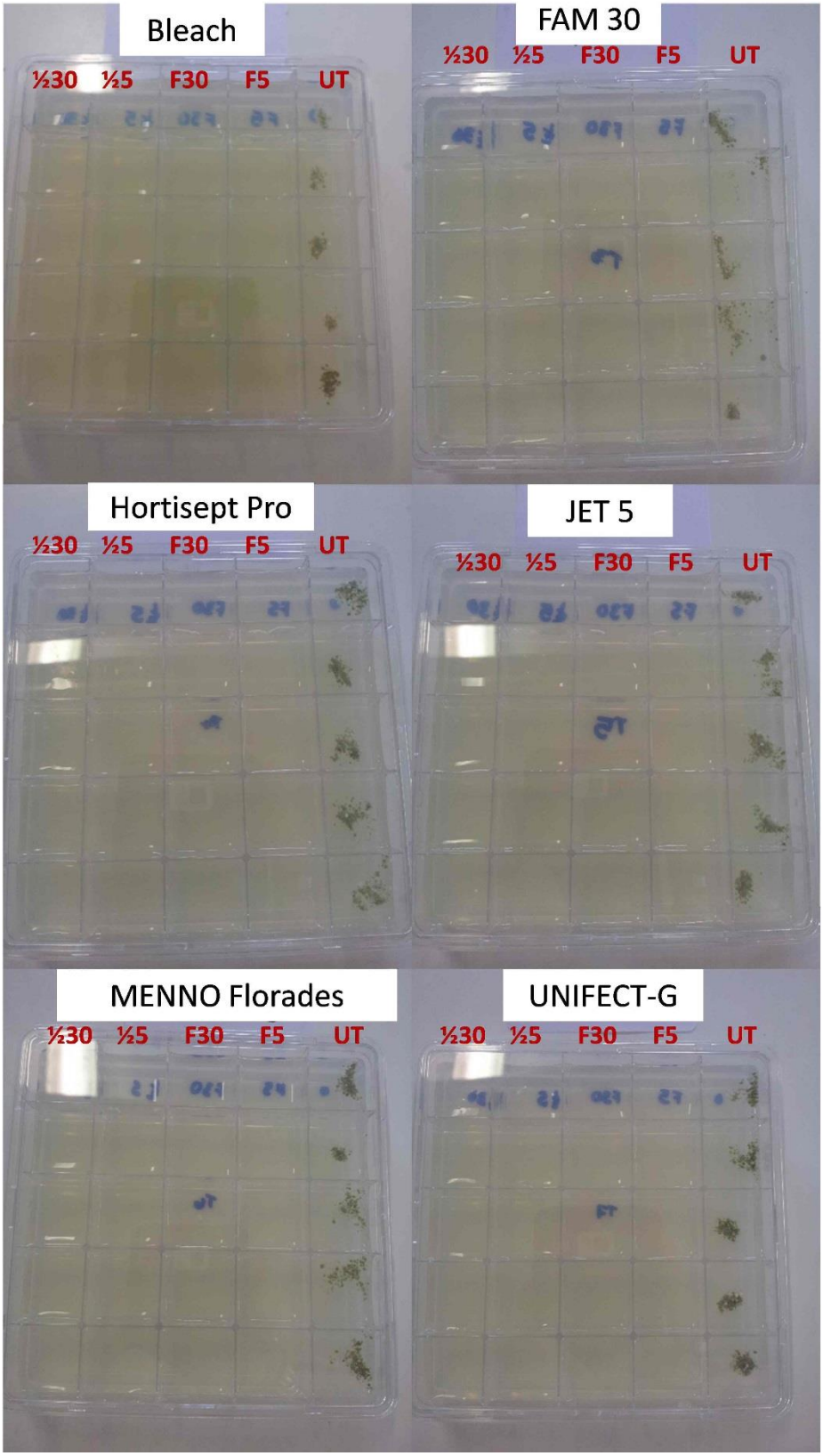

Figure 3. Examples of growth of *P. fulva* (olive green colonies) 7 days after inoculation with disinfectant-treated or untreated (red labelling) spore suspension.

In addition to the main experiment using disinfectants, different hand washing techniques were trialled against spores of *P. fulva* to imitate potential spread by crop workers across nursery sites. Contaminated, unwashed hands spread viable spores that were able to grow on agar plates. On washing with soap and water, or with alcohol gel, no growth of *P. fulva* resulted on the plates although there was some bacterial spread (Fig 4).

Figure 4. Agar plates showing the growth of *P. fulva* (olive green areas) when transferred by hand, and the inhibitory effect of soap and water and alcohol gel on the pathogen.

Experiment 5 – Testing of disinfectants against mycelium of *P. fulva*

When tested against *P. fulva* mycelium grown on filter paper discs, all disinfectant products were 100% effective in preventing growth after 7 days when used at their recommended rate for 30 minutes. However, when used at half rate or for 5 minutes only, products tested varied in their efficacy (Table 19).

Table 19. % fungal growth observed after treatment with six disinfectant products for 5 or 30 minutes, at full or half recommended rates – ADAS Boxworth, 2014 (Experiment 5)

Treatment	Product	Rate (recommended)	Timing (minutes)	% fungal growth (out of 5 wells)
2.	Bleach	0	0	100
		Half	5	80
		Half	30	0
		Full	5	20
		Full	30	0
3.	FAM 30	0	0	100
		Half	5	80
		Half	30	100
		Full	5	80
		Full	30	0
4.	Hortisept Pro	0	0	100
		Half	5	60
		Half	30	20
		Full	5	0
		Full	30	0
5.	JET 5	0	0	100
		Half	5	60
		Half	30	0
		Full	5	0
		Full	30	0
6.	MENNO Florades	0	0	100
		Half	5	20
		Half	30	0
		Full	5	20
		Full	30	0
7.	Unifect-G	0	0	100
		Half	5	20
		Half	30	0
		Full	5	0
		Full	30	0

When tested against mycelium, FAM 30 was the least effective, only achieving a full kill of *P. fulva* when used at the recommended rate for 30 minutes. The most effective product against mycelium was UNIFECT-G, with only 20% fungal growth even when only used at half rate for 5 minutes. JET 5, MENNO Florades and bleach also fully controlled growth of *P. fulva* when used at either half or full rate for 30 minutes. JET 5 was also effective in killing mycelium of *P. fulva* after 5 minutes exposure when used at its full rate. Exposure to MENNO Florades or bleach for only 5 minutes at any rate was not sufficient to kill all *P. fulva* present. Hortisept Pro was effective when used at its full rate for either 5 or 30 minutes, but ineffective for each

exposure time when the rate was halved (Figure 5). Note that only growth emerging from discs was scored as viable *P. fulva*. Some discs appear dark in colour despite being scored as no growth as dead mycelium remained ingrained in the filter paper.

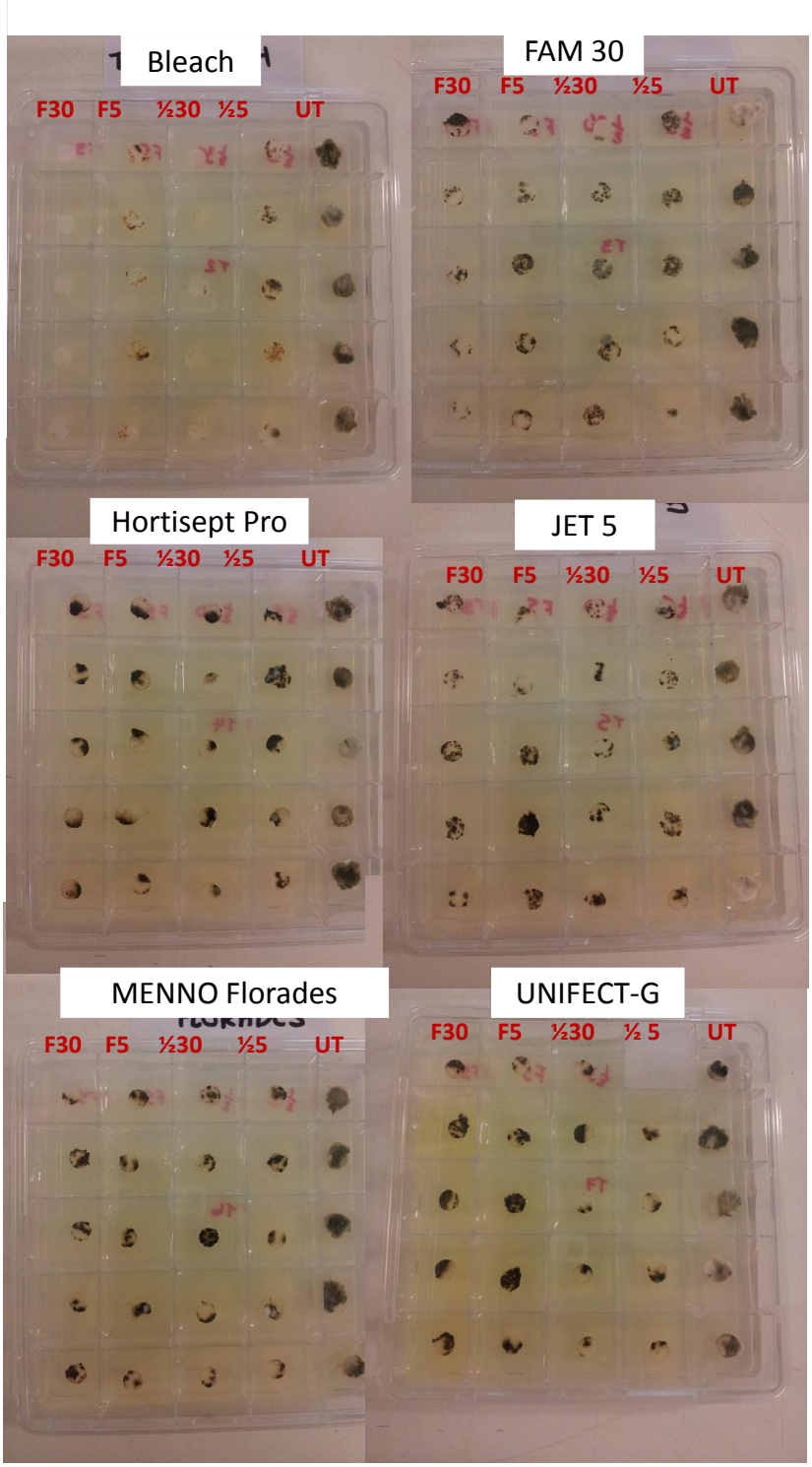

Figure 5. Growth of *P. fulva* (olive green growth emerging from filter paper discs) 7 days after disinfection treatments (red labelling).

Experiment 6 – Testing of disinfectants on a variety of surfaces commonly found on nursery against spores of *P. fulva*

When *P. vulva* was applied to a variety of common surfaces (concrete, glass, plastic, aluminium) which were subsequently treated with disinfectant products, *P. fulva* failed to grow even on the untreated control plates (Table 18). This shows the inoculation technique to be unsuccessful. There were, however, differences in the other fungal and bacterial growth that resulted (Tables 20- 23).

Table 20. Efficacy of disinfectants on a variety of common nursery surfaces against *P. fulva*

	% streaks with *P. fulva* growth on:			
Treatment	Concrete	Glass	Plastic	Aluminium
1. Untreated	0	0	0	0
2. Bleach	0	0	0	0
3. FAM 30	0	0	0	0
4. Hortisept Pro	0	0	0	0
5. Jet 5	0	0	0	0
6. Menno Florades	0	0	0	0
7. Unifect-G	0	0	0	0

Table 21. Efficacy of disinfectants on a variety of common nursery surfaces against fungal growth

	% streaks with fungal growth on:			
Treatment	Concrete	Glass	Plastic	Aluminium
1. Untreated	80	80	50	80
2. Bleach	0	0	20	0
3. FAM 30	30	0	0	0
4. Hortisept Pro	0	0	40	10
5. Jet 5	30	40	10	40
6. Menno Florades	30	40	0	0
7. Unifect-G	0	0	0	10

Table 22. Efficacy of disinfectants on a variety of common nursery surfaces against bacterial growth

	% streaks with bacterial growth on:			
Treatment	Concrete	Glass	Plastic	Aluminium
1. Untreated	100	100	80	80
2. Bleach	20	0	20	0
3. FAM 30	50	0	0	0
4. Hortisept Pro	10	0	10	10
5. Jet 5	10	10	10	30
6. Menno Florades	20	30	0	0
7. Unifect-G	10	10	0	10

Table 23. Efficacy of disinfectants on a variety of common nursery surfaces against bacterial and fungal growth

	% streaks with zero growth on:			
Treatment	Concrete	Glass	Plastic	Aluminium
1. Untreated	0	0	20	20
2. Bleach	80	100	80	100
3. FAM 30	50	100	100	100
4. Hortisept Pro	90	100	60	90
5. JET 5	70	60	80	40
6. MENNO Florades	60	40	100	100
7. Unifect-G	90	90	100	90

Results show that some surfaces are more easily disinfected than others, with higher rates of total disinfection achieved on the smoother surfaces of glass, plastic and aluminium. Concrete was more difficult to disinfect efficiently, though products differed in their efficacy across surfaces. Unifect-G appeared to inhibit both fungal and bacterial growth most effectively across a variety of surfaces.

Experiment 7 – Testing of disinfectants on *P. fulva* infected leaf debris by subsequent transmission testing on glasshouse tomato

First disease symptoms were noted on the 10th September, 12 days after inoculation. Symptoms at the first assessment were not severe, with no sporulation under the leaf and very faint leaf spots appearing above the leaf (Figure 6).

Figure 6. Left - Mycelial growth on the lower leaf surface. Right – Faint leaf spots visible on upper leaf surface, due to infection by *P. fulva*

At the first assessment, all disinfectant treatments succeeded in reducing disease transmission by infected leaf debris when compared to inoculation with untreated leaf debris (Table 24).

Table 24. Effect of disinfectant treatment of infected leaf debris on transmission of *P. fulva* – ADAS Boxworth, 2014

Treatment	% leaf area affected		% area affected inoculated leaf layer	
	12 Sep	19 Sep	12 Sep	19 Sep
1. Untreated	3.8	13.0	14.3	26.3
2. Jet 5	**0.6**	**6.8**	**5.4**	**16.3**
3. Unifect-G	**0.2**	**2.0**	**0.7**	**4.3**
4. Menno Florades	**0.1**	**0.8**	**0.3**	**2.6**
5. Hortisept Pro	**0.1**	**0.4**	**0.2**	**2.0**
P value	<0.001	<0.001	0.001	<0.001
LSD	1.114	3.405	6.117	5.240

Values in bold are significantly different from the untreated.

There were initially no statistical differences between disinfection products. At the final assessment, however, differences between treatments became clearer. When Jet 5 was used to treat leaf debris, significantly higher disease levels were observed than when debris was treated with the other disinfectant products. There were no statistical differences in efficacy between Hortisept Pro, Menno Florades and Unifect-G.

In terms of crop vigour, some positional affects were observed, with plants on the right hand side of the glasshouse growing slightly less vigorously than plants on the left. There were no significant differences in crop vigour between treatments.

Discussion

A link between high relative humidity and disease development is known for *P. fulva*. Notably, high humidity is required for spore production, as observed in Experiment 1. In Experiment 1, no sporulation was observed on the leaf lesions, whereas in Experiment 2, where conditions were more humid in the glasshouses (Appendix 1), dark brown sporulation was observed on the underside of leaves. Any glasshouse management strategies that reduce relative humidity could be expected to contribute effectively to leaf mould management.

In Experiments 2 and 3, Amistar and Switch had the highest efficacy. Amistar was perhaps more consistent in its effect than Switch. Both Signum and Teldor also successfully reduced disease when applied at certain times relative to inoculation. The differences in control achieved by test products between the two experiments may be due to differing levels of inoculum pressure. In Experiment 2, at the final assessment, the leaf area affected on the inoculated leaf layer was 22%, whereas in Experiment 3 this reached 55.6%. For a purely preventative product, when disease has established, it may become difficult to slow its spread. Switch and Amistar, both appeared to have curative action against *P. fulva*, and so may have been able to cope better with increased disease pressure. The cyprodinil in Switch is known for its systemic activity, and the azoxystrobin in Amistar is strongly translaminar, which may explain their efficacy against *P. fulva* already established within the leaves. Teldor is only systemic very locally, and largely works by contact action.

The biological products did not perform as well as the conventional fungicides, but some significant effects on severity and incidence were observed. In Experiment 2, with lower disease pressure, all biological products resulted in disease levels significantly lower than the untreated at the first assessment. In Experiment 3 where disease pressure was higher, two sprays of Serenade ASO and F185 gave significant reductions, though were considerably less effective than a single spray of either Amistar or Switch. Products performed better when used preventatively, especially Serenade ASO, but control tended to drop with time since treatment. Serenade ASO was also successful in reducing incidence of disease in Experiment 2 significantly, and it is likely that this product could be effective if used as part of a preventative programme with other conventional chemicals, particularly when disease pressure is low. HDC F186 was the least effective biological product, giving control comparable to the other two initially, but seeming to have lower persistence. Efficacy of biological products when used preventatively may be due in part to induced resistance in the plant, especially in the case of microbials. If this is so with tomato leaf mould, possibly a greater number of treatment applications, or a longer interval between treatment and inoculation, may have increased the efficacy of biological treatments. Further work would be required to determine if the biological treatments used here induce resistance to leaf mould.

As *P. fulva* infects and sporulates on the lower leaf surface, it was inoculated there directly. The biological products were applied as were the conventional products, in an overhead spray, which may not have achieved good enough coverage of the lower leaf surface for biological products to perform well.

For effective management of tomato leaf mould the products known to have good efficacy should be built into a spray programme according to label restrictions and involving alternating fungicides in different active groups to avoid the development of resistance. Though less effective than Switch, Signum and Amistar, over the course of a season it may be necessary to include Teldor and Serenade ASO in programmes due to the limitations on application numbers/maximum dose limits. The products with good efficacy against *P. fulva* are also known to have good efficacy against *Botrytis cinerea* (with off-label approval for this target in place for Amistar, Switch, Signum, Serenade ASO and Teldor, and an on-label approval for Prestop) and so spray programmes developed to control both diseases would likely be beneficial to growers.

Finally, the results of disinfectant testing will be discussed. Results from tests on different surfaces were uninformative as *P. fulva* failed to grow on plates from swabs taken from disinfected surfaces. Rather than all the disinfectant products tested having 100% efficacy, it is more likely that *P. fulva* spores were overgrown by contaminants, as it is very slow growing on agar. Results against spores of *P. fulva* in solution showed all products to be effective when used at their full recommended rate for 30 minutes. Unlike the surface experiments, these tests were successful as 100% of untreated control treatments yielded *P. fulva* growth. The products that performed best were FAM 30 and bleach, though all products were very effective. When tested against mycelium, more difficult to destroy than spores, all products were again effective when used at full recommended rates for 30 minutes. Based upon these results, using half rates of disinfectant products would not be recommended, and the maximum contact time of product and contaminated surface achievable would be advised. A small experiment on the effect of hand washing also showed that even washing hands with soap and water was highly effective in stopping subsequent growth of *P. fulva*, and so regular hand washing would be recommended for crop workers in a susceptible crop.

When effective products commonly used in the horticultural industry were carried forward to transmission tests, it was seen that debris treated with any of the products resulted in significantly less disease development when used as inoculum compared to untreated debris. This shows that in a commercial situation, effective crop clean up and glasshouse disinfection can be key to lowering the viable inoculum present to infect next year's new crop.

Conclusions

1. Tomato leaf mould disease development was shown to be highly dependent on a high relative humidity, and the maintenance of this high humidity for longer periods increases risk of disease development. Additionally, a higher number of spores inoculated also resulted in a greater incidence of initial disease symptoms.
2. The most effective fungicide treatments against *Passalora fulva* were observed to be Amistar and Switch and the most effective biofungicide treatments were Serenade ASO and HDC F185. Conventional fungicides tested had greater efficacy than any of the biofungicides tested.
3. All disinfectants tested were effective when used at full rate. The most effective treatments against *P. fulva* were Hortisept Pro, Unifect-G and Menno Florades, which worked well against both spores and mycelium *in vitro*, and reduced pathogen transmission.

Knowledge and Technology Transfer

- Mayne S & O'Neill TM (2014). Tomato leaf mould revisited. *AHDB Grower* 209, 22-23.
- Securing plant protection products for sustainable tomato production. AHDB Horticulture/TGA Tomato Conference, Kenilworth, 25 September 2014 (Tim O'Neill).

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the short arm of chromosome 1 in tomato. Molecular and General Genetics, 262, (4-5) 839-845.

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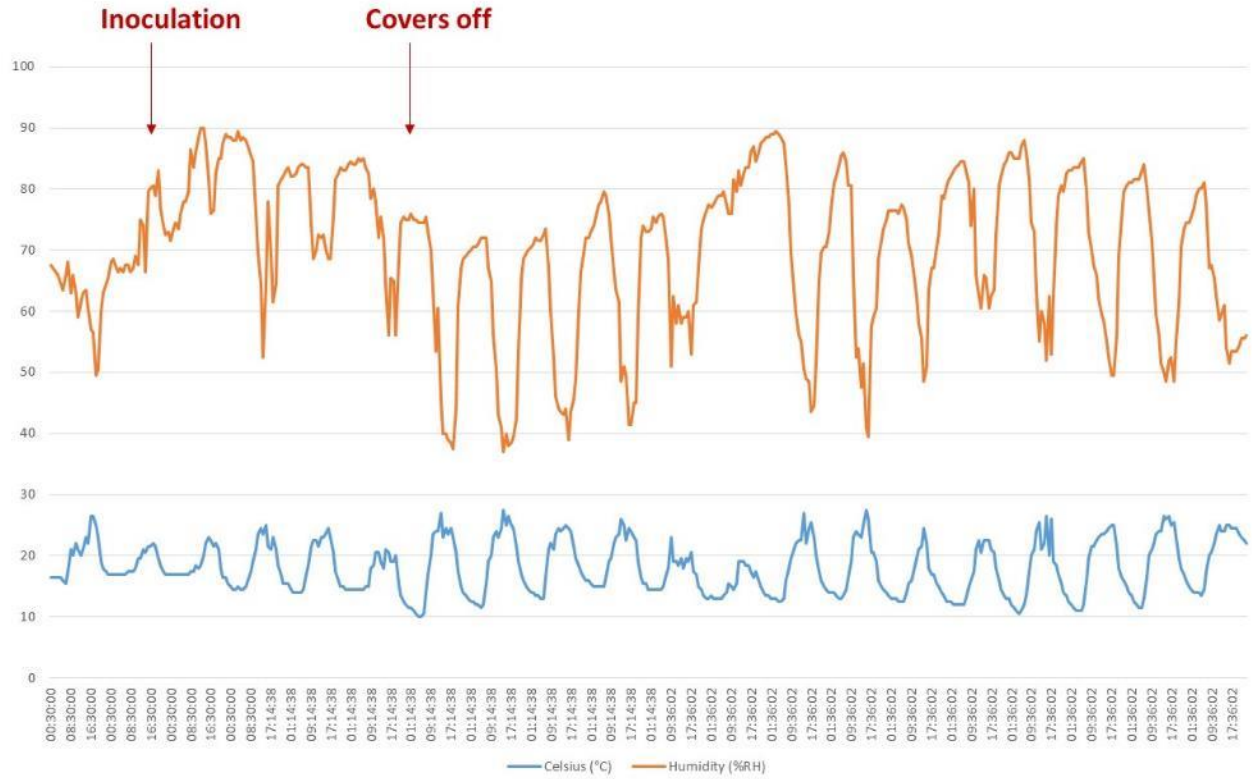
Appendices

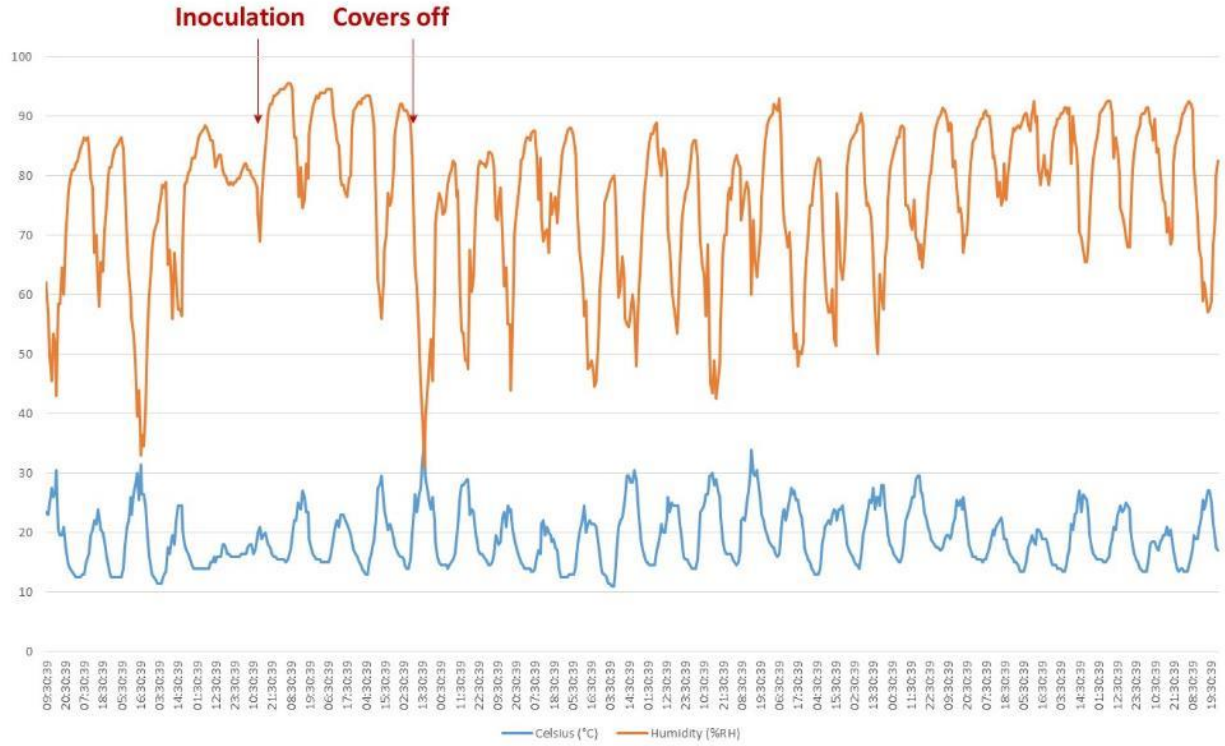
Appendix 1. Temperature and humidity data for glasshouse experiments

Experiment 1

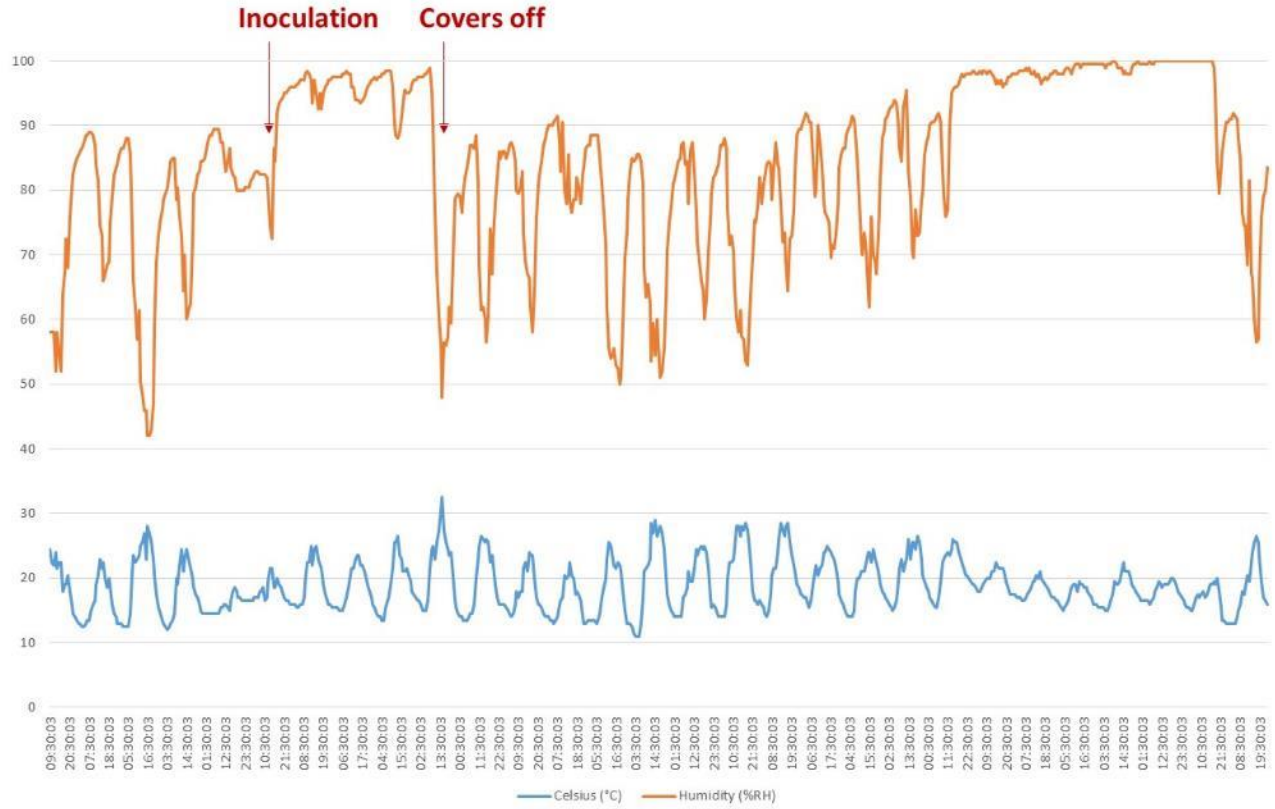


Experiment 2

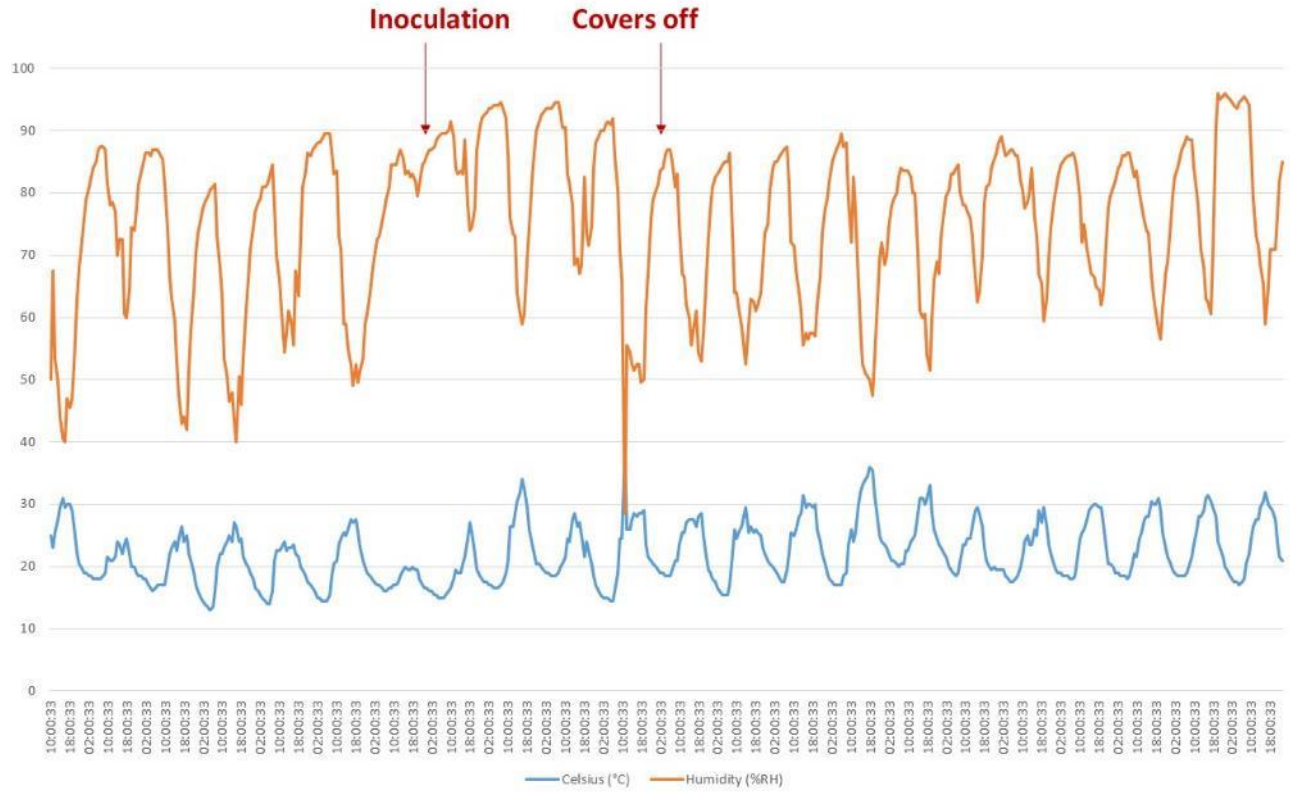
Glasshouse 1



Glasshouse 2



Experiment 3



Experiment 7