SCEPTREPLUS

Final Trial Report

Trial code:	W2018.568 (Glasshouse) and W2018.569 (Polytunnel)
Title:	SP25: Treatments to reduce Fusarium wilt of lettuce
Crop	Group: Field Vegetables – Carrots, lettuce and brassicas
Target	*Fusarium oxysporum* f.sp. *lactucae* race 4 (FOL4)
Lead researcher:	Dr John Clarkson
Organisation:	University of Warwick, School of Life Sciences, Wellesbourne, Warwick CV35 9EF
Period:	June 2018 – February 2019
Report date:	28/02/19
Report author:	John Clarkson
ORETO Number: (certificate should be attached)	381

I the undersigned, hereby declare that the work was performed according to the procedures herein described and that this report is an accurate and faithful record of the results obtained.

28/02/2019	John Clarkson
Date	**Authors signature**

Trial Summary

Introduction

Fusarium wilt of lettuce, caused by *Fusarium oxysporum* f. sp. *lactucae* race 4 (FOL4), first reported in the UK in October 2017 affecting protected lettuce crops in Lancashire and Ireland, represents a significant threat to the UK lettuce industry. A pot-based glasshouse experiment and a polytunnel trial were carried out to identify chemical, and biological treatments to reduce disease caused by FOL4.

Methods

Glasshouse trial

Lettuce plants (cv. Temira, butterhead type) were raised in peat blocks for 16 days with some treatments applied at sowing. Fumigant products were added to FOL4 infested compost in sealed plastic bags and incubated for 2 weeks at 20ºC. Lettuce plants were transplanted into FOL4 inoculated compost in 9 cm square pots, and test products were applied using a knapsack sprayer. Similarly, the fumigated FOL4 compost was also added to individual pots and lettuce transplanted. A total of 24 pots per treatment were placed into a glasshouse compartment in a randomised eight block design with set points of 25°C day / 18°C night, for 6 weeks (July / August 2018). After 23 days (7 days post transplanting) a second spray application was applied to some treatments. Lettuce plants were scored for severity of Fusarium wilt symptoms (0-5 scale) twice weekly and at the end of the trial, plants were cut longitudinally and scored for severity of vascular browning (0-4 scale). The majority of biological treatments were tested as a programme of three applications (at sowing, transplanting and 7 days post-transplanting) while all chemical treatments were tested as a single application at transplanting.

Polytunnel trial

A quarantine polytunnel was inoculated with FOL4 and a lettuce crop grown to confirm disease development and increase the level of inoculum (August / September 2018). Lettuce plants were raised in peat blocks as before and transplanted into the polytunnel soil in plots each containing 12 lettuce plants at 30 cm spacing. Treatments selected based on the results from the glasshouse trial were applied as before with four replicate plots per treatment arranged in a randomised block design. Fusarium wilt symptoms and vascular browning were assessed as above. The trial was carried out October 2018-January 2019.

Results

Artificial inoculation with FOL4 in both glasshouse and polytunnel was successful resulting in a high level of disease and consistent symptoms. For the glasshouse trial, there were statistically significant differences between treatments with Basamid and T34 Biocontrol the most effective, where both Fusarium wilt symptoms and vascular browning were significantly reduced (Table 1). In the polytunnel trial testing Basamid, T34 and Previcur Energy, only Basamid reduced Fusarium wilt as evidenced by a reduction in yellowing leaves, stunting and vascular browning in the lettuce plants (Table 2). There was some phytotoxicity of Basamid as evidenced by a low level of plant mortality soon after transplanting.

Table 1. Effect of biological and chemical fungicide treatments on mean Fusarium wilt and vascular browning symptom scores in lettuce grown in FOL4 inoculated compost in the glasshouse trial.

	Mean Fusarium wilt symptom score (0-5 scale)									**Mean Fusarium vascular browning score (0-4 scale)**
Date	14/08/18	17/08/18	21/08/18	24/08/18	28/08/18	31/08/18	05/09/18	07/09/18	10/09/18	10/09/18
Day	18	21	25	28	32	35	40	42	45	Final
Treatment										
Inoculated control	2.33	2.46	2.75	3.29	3.58	3.63	3.79	4.00	4.25	3.58
Non-inoculated control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.38
Prestop	1.96	2.21	2.38	2.67	3.00	3.04	3.17	3.38	3.42	2.96
Trianum P	2.46	2.88	3.00	3.58	3.79	3.92	4.00	4.17	4.54	3.71
T34	0.88	0.96	0.96	1.13	1.29	1.29	1.33	1.58	1.67	2.50
Amylo X	2.17	2.29	2.58	3.13	3.42	3.46	3.50	3.71	3.88	3.21
Amistar	2.38	2.63	3.00	3.46	3.79	3.96	4.08	4.21	4.50	3.63
Luna Sensation	2.33	2.50	2.96	3.38	3.46	3.54	3.63	3.83	4.17	3.54
Previcur Energy	1.63	1.88	2.13	2.67	2.92	2.96	3.13	3.54	3.88	3.25
Signum	2.42	2.83	3.08	3.46	3.83	3.96	4.13	4.33	4.42	3.54
Switch	2.21	2.46	2.71	3.29	3.71	3.92	4.04	4.29	4.58	3.71
AHDB9896	2.46	2.54	3.00	3.33	3.83	3.92	3.92	4.17	4.50	3.63
Agrichem flowable Thiram	1.88	2.29	2.50	3.17	3.46	3.54	3.79	3.83	4.21	3.42
Inoculated control [1]	1.92	2.08	2.21	2.92	3.17	3.25	3.46	3.79	4.17	3.33
Basamid [1]	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.75
AHDB9895 [1]	3.04	3.38	3.67	4.17	4.50	4.63	4.71	4.83	4.88	3.88
F value	25.17	30.30	33.16	43.48	46.68	52.02	63.71	70.16	84.31	80.39
P -value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
d.f.	105	105	105	105	105	105	105	105	105	105
s.e.d.	0.25	0.25	0.26	0.27	0.28	0.27	0.25	0.25	0.24	0.16
l.s.d.	0.49	0.49	0.52	0.53	0.55	0.54	0.50	0.50	0.48	0.32

Significantly lower than inoculated control

Significantly greater than inoculated control

Table 2. Effect of treatments on the mean number of wilting / yellowing leaves per plant, the percentage of stunted plants, and Fusarium vascular browning score at harvest in the polytunnel trial.

Assessment	Mean number of wilting / yellowing leaves	Mean vascular browning score (0-4)	Mean percentage stunted plants	
Date	19/12/18	11/01/19	19/12/18	
Day	69	92	69	
Treatment			Angular transformed data	Back transformed data
Untreated control	1.60	2.00	20.44	12.19
Previcur Energy	2.04	1.91	21.02	12.87
T34 x 2	0.96	1.69	4.19	0.54
Basamid	0.13	0.00	10.22	3.15
T34 x 3	1.76	1.99	27.05	20.68
F value	8.66	47.71	3.36	
P -value	0.002	<0.001	0.046	
d.f.	12	12	12	
s.e.d.	0.367	0.176	7.10	
l.s.d.	0.800	0.383	15.47	

Significantly lower than the inoculated control

Conclusions

- Single applications of the chemical fungicides tested in the glasshouse trial were not adequate for control of FOL4 while three applications of some biological control agents were effective.
- The soil fumigant Basamid was very effective in preventing Fusarium wilt of lettuce in both the glasshouse and polytunnel trials. There was some evidence of phytotoxicity in the polytunnel trial most likely due to inadequate time between application and planting. Note that Basamid is limited to one application every three years.
- The biological control treatment T34 reduced lettuce Fusarium wilt development in the pot-based glasshouse trial but not in the polytunnel trial. However, the latter trial was conducted in cold conditions which mostly likely compromised control efficacy.
- Prestop showed some efficacy for control of FOL4 in the glasshouse trial (not tested in polytunnel trial).
- None of the chemical fungicide treatments reduced lettuce Fusarium wilt with the exception of Previcur Energy in the glasshouse trial. However, this treatment was not as effective as T34 or Prestop and only delayed the development of symptoms.
- Further work is required to establish the conditions under which T34 is most effective and to determine if multiple applications of chemical fungicides could result in control of Fusarium wilt.

Take home message: Basamid and T34 Biocontrol reduced Fusarium wilt of lettuce caused by FOL4 and could be used as part of an integrated control approach.

Objectives

1. To evaluate the effectiveness of conventional and bio-fungicides as foliar sprays or incorporated soil fumigants for the control of FOL4 in an inoculated pot trial in the glasshouse.
2. To evaluate the most effective treatments from the glasshouse trial for control of FOL4 under semi-commercial conditions in a polytunnel trial.
3. To monitor treated plants for phytotoxicity.

Trial conduct

UK regulatory guidelines were followed but EPPO guidelines took precedence. The following EPPO guidelines were followed:

Relevant EPPO guideline(s)		**Variation from EPPO**
PP 1/152(3)	Design and analysis of efficacy evaluation trials	None
PP 1/135(3)	Phytotoxicity assessment	None
PP 1/181(3)	Conduct and reporting of efficacy evaluation trials including GEP	None

There were no deviations from EPPO guidance.

W2018.568: Glasshouse Trial

Test site

Item	**Details**
Location address	University of Warwick Wellesbourne Campus Wellesbourne Warwick CV35 9EF
Crop	Lettuce
Cultivar	Temira
Soil or substrate type	Levington M2
Agronomic practice	N/A
Prior history of site	N/A

Trial design

Item	**Details**
Trial design:	Randomised Block
Number of replicates:	24 pots per treatment arranged in 8 blocks
Row spacing:	N/A
Plot size: (w x l)	9 cm pots
Plot size: (m^2)	N/A
Number of plants per plot:	N/A
Leaf Wall Area calculations	N/A

Treatment details

AHDB Code	Active substance	Product name/ manufacturer code	Formulation batch number	Content of active substance in product	Formulation type[2]	Adjuvant
	Untreated, Inoculated control					
	Untreated, Non-inoculated control					
Approved	*Gliocladium catenulatum*	Prestop	SAL2561377	32% (2 x 10^8 spores g^{-1})	WP	
Approved	*Trichoderma harzianum*	Trianum P	18TP19	1% (1 x 10^9 spores g^{-1})	WP	None
Approved	*Trichoderma asperellum*	T34	18171619	12% (1 x 10^{12} cfu g^{-1})	WP	None
Approved	*Bacillus amyloliquefaciens*	Amylo X	2581637	250 g/kg (5 x 10^{13} cfu g^{-1})	WG	None
Approved	Azoxystrobin	Amistar	GRA8A00005	250 g L^{-1}	SC	None
Approved	Fluopyram + Trifloxystrobin	Luna Sensation	NK44GX1946	250 g/l + 250 g L^{-1}	SC	None
Approved	Propamocarb + Fosetyl	Previcur Energy	EM4L023621	530 g/l +310 g L^{-1}	SL	None
Approved	Boscalid + pyraclostrobin	Signum	12-P00528	26.7% + 6.7%	SC	None
Approved	Cyprodinil + Fludioxonil	Switch	CHE7E60076	37.5% + 25%	WG	None
AHDB9896	Garlic extract	0021		100%	Liquid	None
Approved	Thiram	Agrichem flowable Thiram	36420	600 g L^{-1}	Suspension	None
	Untreated, Inoculated control [1]					
Approved	Dazomet [1]	Basamid[1]	28991688Q0	97%	G	None
AHDB9895	*B. carinata* [1]	Biofence[1]				None

[1] Incubated for 2 weeks with *Fusarium* infested compost at room temperature before using in glasshouse trial (for comparison with fumigant products).

[2] WP, wettable powder; WG, wettable granule; SL, suspension liquid; SC, suspension concentrate

Glasshouse Trial: Methods, assessments and records

The overall approach to test products for control of FOL4 in glasshouse pot tests was to i) apply fumigant treatments to compost infested with the pathogen pre-planting (Basamid, AHDB9895; application A), ii) apply biological products / thiram at sowing directly to seed (application B1, B2), iii) apply biological and chemical fungicides at transplanting of lettuce into FOL4 infested compost (application C1, C2, C3) and iv) apply biological products 7 days post-transplanting (application D). Hence, biological products were tested as a program of three applications (at seeding, at transplanting and 7 days post-transplanting; with exception of Trianum; two applications B1, C1) while chemical fungicides were applied once at transplanting and the botanical AHDB9896 applied twice (C2, D) These application programmes were adopted following consultation with the relevant crop protection companies and AHDB.

Fumigant products were applied at the appropriate rate to 1kg compost infested with FOL4 (1×10^4 cfu g^{-1}) contained in 2L sealable plastic bags on 04/07/18. The moisture content of the compost was raised to 77% (w/w) by the addition of 323 ml water per bag. There were eight replicate bags per treatment which were sealed and incubated for two weeks at 20ºC. At the end of this period, the bags were opened and vented for 7 days to allow escape of remaining volatiles after which a cress phytotoxicity test was conducted to confirm lack of phytotoxic residues. An inoculated control treatment was also set up for comparison (FOL4 infested compost only). Actively growing fungal mycelium was observed in the AHDB9895 treated compost one week after incubation.

Untreated pelleted lettuce seed (cv. Temira, butterhead type) were sown into peat blocks (4.5 x 4.5 x 4.5 cm) on 11/07/18. Biological treatments applied at this propagation stage (application B1 / B2) were pipetted on the surface of the block around the seed. Control treatments received water only in the same volume. After 16 days, lettuce from the treated and untreated peat blocks were transplanted into compost freshly inoculated with FOL4 (1×10^4 cfu g^{-1}) in square 9 cm pots (FP9). The moisture content of the compost was raised to 77% by the addition of 80 ml water per pot. All biological and chemical treatments were applied at this stage (applications C1, C2; 27/07/18) using a knapsack sprayer fitted with a 05F110 nozzle. Similarly, the fumigated FOL4 compost treatments were also added to individual pots and an untreated lettuce transplant added. After 23 days (7 days post transplanting, 03/08/18) a second spray application was applied for biological treatments only (application D). An untreated control treatment (infested compost only) was also set up.

In total there were 24 replicate pots per treatment arranged in a randomized eight block design in a glasshouse compartment and conditions were set at 25°C day / 18°C night with a 16 hour day length, for 6 weeks.

Application schedule

	Treatment: product name or AHDB code	Rate of active substance (ml or g a.s./ha)	Rate of product (l or kg/ha)	Application code
1	Inoculated control			
2	Non-inoculated control			
3	Prestop	32000 g	100 kg (100 @ B1 = 40.6 mg/block)	B1 C1 D
4	Trianum P	1500 g	150 kg (15 @ B1 = 6.09 mg/block)	B1 C1
5	T34	12000 g	100 kg (100 @ B1 = 40.6 mg/block)	B1 C1 D
6	Amylo X	625 g	2.5 kg (1.02 @ B1 = 40.6 mg/block)	B1 C1 D
7	Amistar	250 g	1 L	C2
8	Luna Sensation	Fluopyram 200 g Trifloxystrobin 200 g	0.8 L	C2
9	Previcur Energy	Propamocarb 1325 g Fosetyl 775	2.5 L	C2
10	Signum	Boscalid 200.25 g Pyraclostrobin 502.5 g	0.75 L	C2
11	Switch	Cyprodinil 225 g Fludioxonil 150 g	0.6 kg	C2
12	AHDB9896		300 L	C2 D
13	Agrichem flowable Thiram	366 g	0.61 L [2]	B2
14	Inoculated control [1]			
15	Basamid [1]	737 kg	760 kg	A
16	AHDB9895 [1]		1000 kg	A

[1] Incubated for 2 weeks at room temperature in FOL infested compost
[2] Recommended rate 5 L /1000 kg seed, 900,000 seeds/kg, 110,000 plants/ha

Application details

	Application A	Application B1	Application B2	Application C1	Application C2	Application D
Application date	04/07/18	11/07/18	11/07/18	27/07/18	27/07/18	03/08/18
Time of day	13.30	10.30	10.30	13.30	15.30	10.30
Crop growth stage (Max, min average BBCH)	N/A	Sowing	Sowing	14	14	15
Crop height (cm)	N/A	N/A	N/A	5	5	7
Crop coverage (%)	N/A	N/A	N/A	20	20	30
Application Method	Incorporation	Drench	Drench	Spray	Spray	Spray
Application Placement	Compost	Seed	Seed	Foliar	Foliar	Foliar
Application equipment	Beaker	Pipette	Pipette	Berthoud Vermorel 2000HP	Berthoud Vermorel 2000HP	Berthoud Vermorel 2000HP
Nozzle pressure	N/A	N/A	N/A	2 bar	2 bar	2 bar
Nozzle type	N/A	N/A	N/A	05F110	05F110	05F110
Nozzle size	N/A	N/A	N/A	05	05	05
Application water volume/block	N/A	2.03 ml	0.1 ml	10,000l [1]	1000l	10,000l [1]
Temperature of air - shade (°C)	N/A	N/A	N/A	27 [2]	27 [2]	24 [2]
Relative humidity (%)	N/A	N/A	N/A	N/A	N/A	N/A
Wind speed range (m/s)	N/A	N/A	N/A	N/A	N/A	N/A
Dew presence (Y/N)	N/A	N/A	N/A	N/A	N/A	N/A
Temperature of soil - 2-5 cm (°C)	N/A	N/A	N/A	N/A	N/A	N/A
Wetness of soil - 2-5 cm	N/A	N/A	N/A	N/A	N/A	N/A
Cloud cover (%)	N/A	N/A	N/A	N/A	N/A	N/A

[1] AHDB9896 – 5000l/ha
[2] Outdoor temperature for duration of spraying only

Untreated levels of pests/pathogens at application and through the assessment period

Common name	Scientific Name	EPPO Code	Disease level pre-application	Disease level at start of assessment period	Disease level at end of assessment period
Fusarium	*Fusarium oxysporum* f.sp. *lactucae* race 4 (FOL4)		0% incidence	100% incidence in inoculated control with mean wilting score of 2.3	100% incidence in inoculated control with mean wilting score of 4.3 (max =5)

Assessment details

All lettuce plants were scored for disease symptoms twice weekly using a scoring system based on severity of disease expression (0 - 5 scale; Table 3). At the end of the experiment (10/09/19), individual lettuce heads were cut in half longitudinally through the root and scored for severity of internal vascular browning (0 - 4 scale; Table 3)

Table 3. Lettuce Fusarium disease symptom scores

Score	Plant symptoms	Vascular browning symptom
0	Healthy	No symptoms
1	1-2 leaves wilting (<10%)	Mild vascular browning
2	10-50% wilted	Moderate vascular browning
3	>50% wilted	Severe Vascular browning
4	Total wilt	Dead/total browning
5	Dead	

Evaluation date	Evaluation Timing (DA)*	Crop Growth Stage (BBCH)	Evaluation type (efficacy, phytotox)	Assessment
3/8/18	7	15	Phytotoxicity	Leaf damage
14/8/18	18	17	Efficacy	Wilting leaf score
17/8/18	21	17	Efficacy	Wilting leaf score
21/8/18	25	18	Efficacy	Wilting leaf score
24/8/18	28	18	Efficacy	Wilting leaf score
28/8/18	32	33	Efficacy	Wilting leaf score
31/8/18	35	35	Efficacy	Wilting leaf score
5/9/18	40	37	Efficacy	Wilting leaf score
7/9/18	42	37	Efficacy	Wilting leaf score
10/9/18	45	39	Efficacy	Wilting leaf score
10/9/18	49	39	Efficacy	Vascular browning score

* DA – days after transplanting / first spray application

Statistical analysis

The trial was analysed by Andrew Mead at Rothamsted Research as a randomised complete block design with 4 replicates of 16 treatments, using ANOVA using the Genstat program. No data transformation was required.

Glasshouse Trial: Results

Phytotoxicity

There was no evidence of phytotoxic effects for any of the treatments.

Effect of treatments on FOL4 disease development

Several treatments had a significant ($p<0.05$) effect both on the severity of Fusarium wilt symptoms on lettuce at the different timepoints (Table 3, Fig. 1) and also on the severity of vascular browning when plants were dissected at the end of the experiment (Table 4, Fig. 2). The Basamid treatment in particular resulted in no visible wilting symptoms at any of the timepoints and a very low mean vascular browning score (0.750) that was only marginally greater than the uninoculated control (0.375).

Two biological products (three applications), T34 Biocontrol and Prestop, resulted in reduced severity of wilting with T34 the most effective with a mean disease score of 1.667 at the last assessment (10/09/18, 45 days after planting) compared with 4.250 for the inoculated control (Table 4, Fig. 1). These treatments also significantly reduced vascular browning with T34 resulting in a mean score of 2.500 compared with 3.583 for the inoculated control (Table 4, Fig. 2).

None of the chemical fungicide treatments (single application) resulted in control of Fusarium wilt with the exception of Previcur Energy which initially significantly reduced wilting at time points up to 40 days after planting, and also slightly (but significantly) reduced vascular browning at the end of the experiment (mean score 3.250).

The fumigant AHDB9895 appeared to increase Fusarium wilt, most likely by providing a substrate for further growth during the incubation period resulting in an increased FOL4 inoculum level at transplanting.

Table 4. Effect of biological and chemical fungicide treatments on mean Fusarium wilt in lettuce grown in FOL4 inoculated compost in the glasshouse trial.

	Mean Fusarium wilt symptom score (0-5 scale)									**Mean Fusarium vascular browning score (0-4 scale)**
Date	14/08/18	17/08/18	21/08/18	24/08/18	28/08/18	31/08/18	05/09/18	07/09/18	10/09/18	10/09/18
Day	18	21	25	28	32	35	40	42	45	Final
Treatment										
Inoculated control	2.33	2.46	2.75	3.29	3.58	3.63	3.79	4.00	4.25	3.58
Non-inoculated control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.38
Prestop	1.96	2.21	2.38	2.67	3.00	3.04	3.17	3.38	3.42	2.96
Trianum P	2.46	2.88	3.00	3.58	3.79	3.92	4.00	4.17	4.54	3.71
T34	0.88	0.96	0.96	1.13	1.29	1.29	1.33	1.58	1.67	2.50
Amylo X	2.17	2.29	2.58	3.13	3.42	3.46	3.50	3.71	3.88	3.21
Amistar	2.38	2.63	3.00	3.46	3.79	3.96	4.08	4.21	4.50	3.63
Luna Sensation	2.33	2.50	2.96	3.38	3.46	3.54	3.63	3.83	4.17	3.54
Previcur Energy	1.63	1.88	2.13	2.67	2.92	2.96	3.13	3.54	3.88	3.25
Signum	2.42	2.83	3.08	3.46	3.83	3.96	4.13	4.33	4.42	3.54
Switch	2.21	2.46	2.71	3.29	3.71	3.92	4.04	4.29	4.58	3.71
AHDB9896	2.46	2.54	3.00	3.33	3.83	3.92	3.92	4.17	4.50	3.63
Agrichem flowable Thiram	1.88	2.29	2.50	3.17	3.46	3.54	3.79	3.83	4.21	3.42
Inoculated control [1]	1.92	2.08	2.21	2.92	3.17	3.25	3.46	3.79	4.17	3.33
Basamid [1]	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.75
AHDB9895 [1]	3.04	3.38	3.67	4.17	4.50	4.63	4.71	4.83	4.88	3.88
F value	25.17	30.30	33.16	43.48	46.68	52.02	63.71	70.16	84.31	80.39
P -value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
d.f.	105	105	105	105	105	105	105	105	105	105
s.e.d.	0.25	0.25	0.26	0.27	0.28	0.27	0.25	0.25	0.24	0.16
l.s.d.	0.49	0.49	0.52	0.53	0.55	0.54	0.50	0.50	0.48	0.32

Significantly lower than inoculated control

Significantly greater than uninoculated control

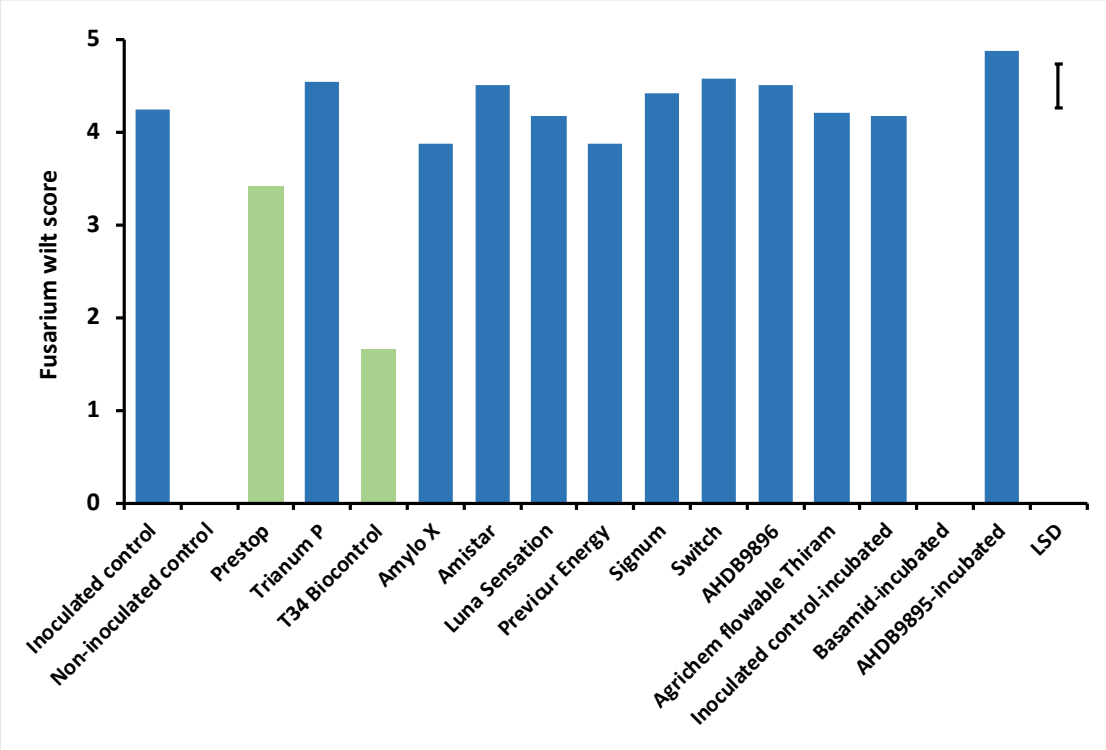


Figure 1. Effect of treatments on mean Fusarium wilt disease score in glasshouse trial 45 days after inoculation. Green bars indicate those significantly different from the inoculated control. LSD = least significant difference (p≤0.05).

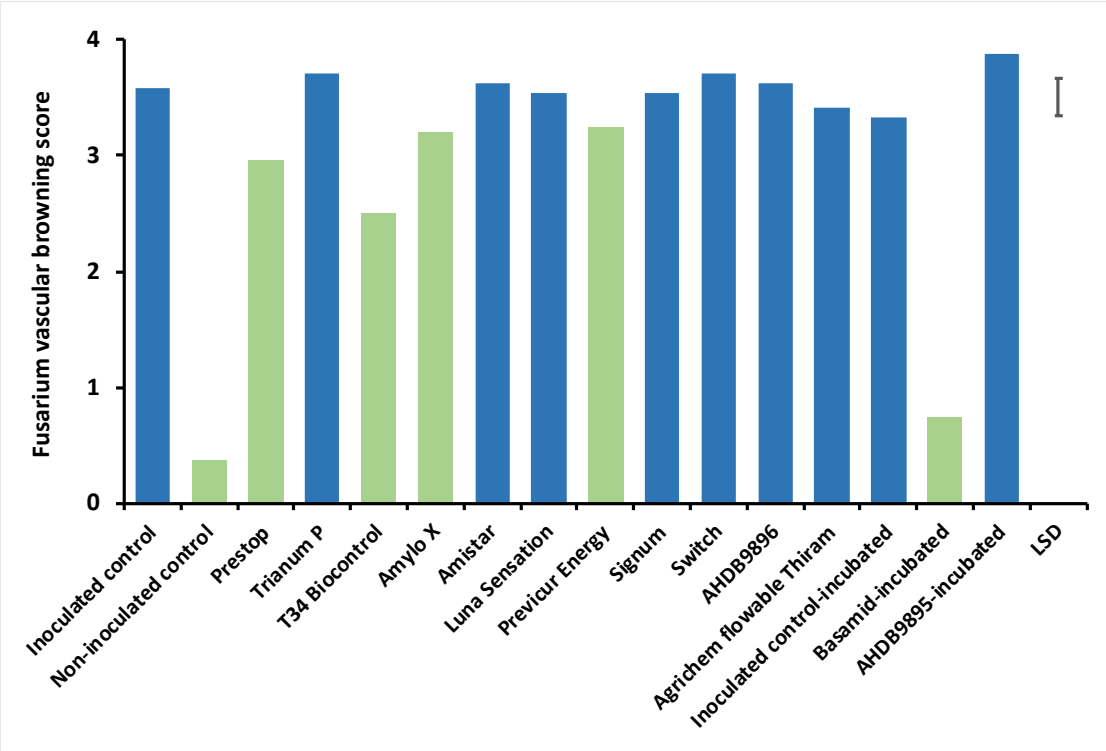


Figure 2. Effect of treatments on mean Fusarium vascular browning disease score in glasshouse trial at harvest. Green bars indicate those significantly different from the inoculated control. LSD = least significant difference (p≤0.05).

W2018.569: Polytunnel Trial

Test site

Item	Details
Location address	University of Warwick Wellesbourne Campus Wellesbourne Warwick CV35 9EF
Crop	Lettuce
Cultivar	Temira
Soil or substrate type	Sandy-loam soil
Agronomic practice	N/A
Prior history of site	Inoculated with FOL 11/5/18 Lettuce crop planted 16/5/18 Crop destroyed and rotavated in 19/9/18

Trial design

Item	Details
Trial design:	Randomized Block
Number of replicates:	4
Row spacing:	30 cm
Plot size: (w x l)	180 x 60 cm
Plot size: (m^2)	1.08
Number of plants per plot:	12
Leaf Wall Area calculations	N/A

Treatment details

AHDB Code	Active substance	Product name/ manufacturer code	Formulation batch number	Content of active substance in product	Formulation type	Adjuvant
	Untreated control					
	Untreated, Non-inoculated control					
Approved	*Trichoderma asperellum*	T34	18171619	12% (1×10^{12} cfu g^{-1})	WP	None
Approved	Propamocarb + Fosetyl	Previcur Energy	EM4L023621	530 g L^{-1} +310 g L^{-1}	SL	None
Approved	Dazomet	Basamid	28991688Q0	97%	G	None

Polytunnel Trial: Methods, assessments and records

This trial was conducted in a polytunnel which had previously been inoculated with FOL4 and lettuce grown to confirm disease development and increase the level of inoculum. This resulted in 100% disease incidence and the infected crop was rotavated (19/9/18) 22 days before this trial was carried out. The trial tested the best fumigant (Basamid), biological control (T34) and chemical fungicide (Previcur Energy) products from the glasshouse trial.

The overall approach to test products in this trial was to i) apply Basamid fumigant treatments pre-planting (application A), ii) apply T34 Biocontrol on three occasions at sowing directly to seed, at transplanting and 7 days after transplanting (application B, C, D) or on two occasions; at transplanting and 7 days after transplanting (application C, D), ii) apply Previcur Energy on two occasions; at transplanting and 7 days after transplanting (application C, D).

Basamid was weighed into containers and sprinkled over marked plots in the polytunnel (20/09/18). The granules were incorporated to a depth of approximately 15 cm using a fork and watered in (10L per plot) to increase the moisture content to approximately 80% of field capacity. The plots were then covered and with a polythene sheet which was dug in to a depth of about 10 cm on all four sides to seal. After two weeks the polythene was removed and the plots allowed to vent for 7 days. The timetable of the trial did not allow for a cress phytotoxicity test to be conducted on this occasion.

Untreated pelleted lettuce seed (cv. Temira, butterhead type) were sown into peat blocks (4.5 x 4.5 x 4.5 cm) on 27/09/18. The T34 treatment applied at this propagation stage (application B) was applied using a pipette to the surface of the block around the seed. After 16 days, lettuce from the treated and untreated peat blocks were transplanted into the polytunnel plots and Previcur Energy and T34 treatments applied using a knapsack sprayer fitted with a 05F110 nozzle (11/10/18). After 23 days (7 days post transplanting, 18/10/18) a second spray application was applied for Previcur Energy and T34 treatments (application D). Untreated control treatments were also set up in the polytunnel.

In total, there was a total of 48 plants for each of the five treatments, each comprising four replicate plots of 12 plants randomised in the FOL4 infested polytunnel. Conditions in the polytunnel depended on ambient conditions and soil temperature was recorded with a logger (Appendix). A propane heater was installed mid-way through the trial to try and elevate the temperature and promote FOL4 disease development.

Application schedule

	Treatment: product name or AHDB code	**Rate of active substance (ml or g a.s./ha)**	**Rate of product (L or kg/ha)**	**Application code**
1	Untreated control			
2	Previcur Energy	Propamocarb 1325 g Fosetyl 775 g	2.5 L	C D
3	T34	12 kg	100 kg (100 @ B1 = 40.6 mg/block)	C D
4	Basamid	737 kg	760 kg	A
5	T34	12 kg	100 kg (100 @ B1 = 40.6 mg/block)	B C D

Application details

	Application A	Application B	Application C	Application D
Application date	20/9/18	20/9/18	11/10/18	18/10/18
Time of day	13.30	10.30	10.30	10.30
Crop growth stage (Max, min average BBCH)	N/A	Sowing	15	16
Crop height (cm)	N/A	N/A	7	7
Crop coverage (%)	N/A	N/A	10	10
Application Method	Incorporation to 15 cm	Drench	Spray	Spray
Application Placement	Soil [2]	Seed	Foliar	Foliar
Application equipment	Beaker	Pipette	Berthoud Vermorel 2000HP	Berthoud Vermorel 2000HP
Nozzle pressure	N/A	N/A	2 bar	2 bar
Nozzle type	N/A	N/A	05F110	05F110
Nozzle size	N/A	N/A	05	05
Application water volume	N/A	2.03 ml/block	10,000 L/ha (T34) [1] 1000 L/ha (Previcur Energy) [1]	
Temperature of air - shade (°C)	25	26	25	20
Relative humidity (%)	N/A	N/A	Not recorded	Not recorded
Wind speed range (m/s)	N/A	N/A	N/A	N/A
Dew presence (Y/N)	N/A	N/A	N/A	N/A
Temperature of soil - 2-5 cm (°C)	Not recorded	N/A	Not recorded	Not recorded
Wetness of soil - 2-5 cm	Approximately 80% field capacity after treatment	N/A	N/A	N/A
Cloud cover (%)	N/A	N/A	N/A	N/A

[1] Applied In 10 cm band over plants
[2] Water applied after application to raise moisture to approximately 80% of field capacity. Plots then covered with polythene which was sealed by digging in edges to 10 cm.

Untreated levels of pests/pathogens at application and through the assessment period

Common name	Scientific Name	EPPO Code	Disease level pre-application	Disease level at start of assessment period	Disease level at end of assessment period
	Fusarium oxysporum f.sp. *lactucae* race 4 (FOL4)		0% incidence	69% incidence in inoculated control	94% incidence in inoculated control with mean vascular browning disease score of 2.0 (max = 4)

Assessment details

When disease symptoms started to occur (19/12/18), the lettuce plants were assessed for severity of Fusarium disease expression by counting the numbers of yellowing/ wilting leaves per plant (as there was no apparent wilting). The number of plants which were stunted was also recorded. However, due to cold conditions and some Botrytis infection (recorded for each plant), symptoms due to FOL4 were not always clear in this assessment. At the end of the trial (11/01/19) individual lettuce heads were cut in half longitudinally through the root and scored for severity of internal vascular browning on a 0 - 4 scale (Table 3).

Evaluation date	Evaluation Timing (DA)*	Crop Growth Stage (BBCH)	Evaluation type (efficacy, phytotox)	Assessment
18/10/18	7	16	Phytotoxicity	Plant death
19/12/18	69	35	Efficacy	Wilting leaf score
11/01/19	92	37	Efficacy	Vascular browning score

* DA – days after first spray application (transplanting)

Statistical analysis

The trial was analysed by Andrew Mead at Rothamsted Research as a randomised complete block design with 4 replicates of 16 treatments, using ANOVA using the Genstat program. No data transformation was required except for percentage plants stunted where an angular transformation was required.

Polytunnel Trial: Results

Phytotoxicity

There was some evidence of phytotoxic effects with the Basamid fumigant treatment. This was seen as plant mortality (Table 5). No other treatments were phytotoxic.

Table 5. Phytotoxicity in lettuce 7 days after planting

Plot	Number of dead plants (Total plants = 12)
4	1
8	1
13	0
19	2

Effect of treatments on FOL4 disease development

None of the treatments tested significantly ($p<0.05$) reduced symptoms of Fusarium wilt (yellow leaves / stunting) in lettuce plants or vascular browning (when plants were dissected at the end of the experiment) with the exception of Basamid (Table 6, Fig. 3). Here, Basamid reduced the numbers of yellowing leaves to 0.13 compared with 1.60 for the untreated control and resulted in no vascular browning at harvest. Basmid also reduced the percentage of stunted lettuce plants but not significantly. Two applications of T34 appeared to significantly decrease stunting compared with the untreated control but three applications did not have the same effect.

Table 6. Effect of treatments on the mean number of wilting / yellowing leaves per plant, the mean percentage of stunted plants, and mean Fusarium vascular browning score at harvest in the polytunnel trial.

Assessment	Mean number of wilting / yellowing leaves	Mean vascular browning score (0-4)	Mean percentage stunted plants	
Date	19/12/18	11/01/19	19/12/18	
Day	69	92	69	
Treatment			Angular transformed data	Back transformed data
Untreated control	1.60	2.00	20.44	12.19
Previcur Energy	2.04	1.91	21.02	12.87
T34 x 2	0.96	1.69	4.19	0.54
Basamid	0.13	0.00	10.22	3.15
T34 x 3	1.76	1.99	27.05	20.68
F value	8.66	47.71	3.36	
P -value	0.002	<0.001	0.046	
d.f.	12	12	12	
s.e.d.	0.367	0.176	7.10	
l.s.d.	0.800	0.383	15.47	

Significantly lower than the inoculated control

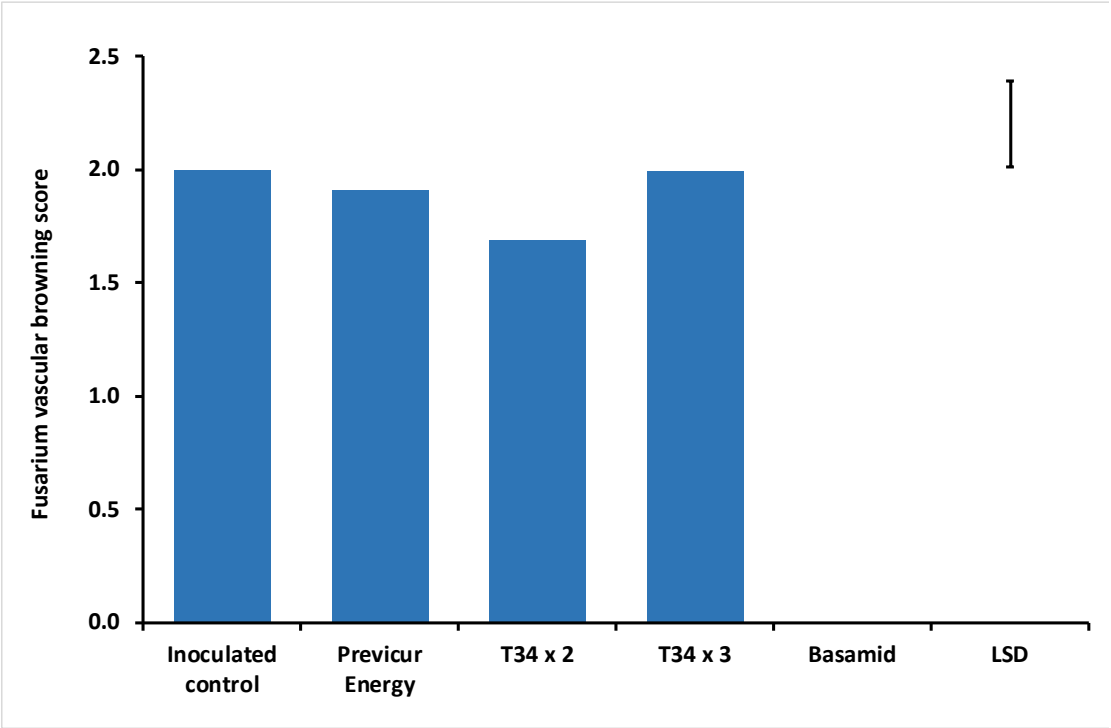


Figure 3. Effect of treatments on mean Fusarium vascular browning disease score in the polytunnel trial. Only the Basamid treatment was significantly different from the inoculated control. LSD = least significant difference (P≤0.05).

Botrytis disease

A small number of plants on both assessment dates were seen with the symptoms of *Botrytis* infection which could have been a secondary infection after infection with FOL4. The percentage plants with *Botrytis* were analysed after angular transformation (Table 7). The analysis revealed that there were no significant differences in Botrytis incidence between treatments (p≤0.05).

Table 7. Percentage of lettuce plants with Botrytis at two assessment dates.

Assessment	**Percentage plants with *Botrytis***			
Date	19/12/18		11/01/19	
Day	69		92	
Treatment	Angular transformed data	Back transformed data	Angular transformed data	Back transformed data
Untreated control	16.78	8.33	19.03	10.64
Previcur Energy	23.23	15.56	22.27	14.36
T34 x 2	8.82	2.35	10.22	3.15
Basamid	14.41	6.20	25.22	18.15
T34 x 3	22.27	14.36	10.22	3.15
F value	1.27		1.97	
P -value	0.336		0.164	
d.f.	12		12	
s.e.d.	7.44		6.96	
l.s.d.	16.21		15.16	

Discussion

Glasshouse trial

Artificial inoculation with FOL4 in the glasshouse pot trial was successful, resulting in a high level of disease and consistent symptoms with 100% incidence in the untreated control at the end of the experiment and clear vascular browning following dissection of lettuce stems (disease score of 3.6; max = 4). There were clear differences between treatments with Basamid completely inhibiting Fusarium wilt development, confirming its effectiveness as a soil fumigant. Of the biological treatments tested in a programme of three applications, T34 Biocontrol reduced wilting symptoms caused by FOL4 confirming its reported activity against *Fusarium* diseases. T34 treated plants were comparable in appearance / quality to those of the untreated control for most of the disease assessments carried out although some symptoms appeared towards the end of the trial. This was evidenced by some vascular browning when lettuce plants were dissected but this was nonetheless significantly reduced compared to the inoculated control. Prestop also showed some activity against FOL4, but was much less effective than T34. None of the chemical fungicides tested as single applications showed any activity against FOL4 with the exception of Previcur Energy. However, although this treatment resulted in statistically significant reductions in Fusarium wilt symptoms early on in the trial, plants subsequently succumbed to the disease and by the end there was only a small (but significant) decrease in vascular browning. The lack of control by any of the chemical fungicide applications may indicate that more than one spray is required for FOL control.

Polytunnel trial

Artificial inoculation of a polytunnel with FOL4 was successful as it resulted in a high level of disease and consistent symptoms with 100% incidence in a previous lettuce crop grown in July / August 2018. Although Fusarium wilt symptoms developed in this trial, they were slow to develop and there was no clear wilting. Assessments were also confounded by Botrytis infections which were most likely due to the suboptimal conditions for lettuce growth and the plants being compromised by FOL4 infection. Although an attempt to mitigate this was made by using a heater to elevate temperature, the very cold ambient conditions meant that overall the trial was not conducted in ideal conditions. Nonetheless, there was again clear evidence that Basamid was effective against FOL4 resulting in a large and significant reduction in vascular browning. There was however some phytotoxic effect as observed by a low level of plant mortality post-transplanting, most likely because inadequate time was available between removal of plastic sheeting and lettuce planting to allow full venting of volatiles. In contrast, neither T34 Biocontrol nor Previcur Energy resulted in any reduction in Fusarium wilt as observed in the glasshouse trial. For T34, one potential reason for this was the cold temperatures which would have reduced its ability to effectively colonise roots as they developed post-transplanting. A further trial under warmer conditions is warranted to test these treatments more effectively.

Conclusions

- Single applications of the chemical fungicides tested in the glasshouse trial were not adequate for control of FOL4 while three applications of some biological control agents were effective.
- The soil fumigant Basamid was very effective in preventing Fusarium wilt of lettuce in both the glasshouse and polytunnel trials. There was some evidence of phytotoxicity in the polytunnel trial most likely due to inadequate time between application and planting. Note that Basamid is limited to one application every three years.
- The biological control treatment T34 reduced lettuce Fusarium wilt development in the pot-based glasshouse trial but not in the polytunnel trial. However, the latter trial was conducted in cold conditions which mostly likely compromised control efficacy.
- Prestop showed some efficacy for control of FOL4 in the glasshouse trial (not tested in polytunnel trial).
- None of the chemical fungicide treatments reduced lettuce Fusarium wilt with the exception of Previcur Energy in the glasshouse trial. However, this treatment was not as effective as T34 or Prestop and only delayed the development of symptoms.
- Further work is required to establish the conditions under which T34 is most effective and to determine if multiple applications of chemical fungicides could result in control of Fusarium wilt.

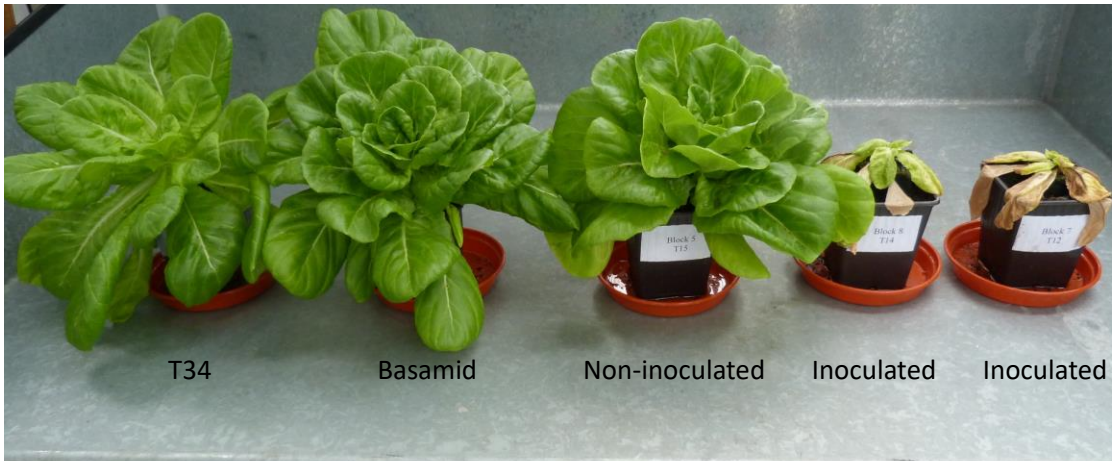
Acknowledgements

We would like to thank Andrew Mead for statistical support and AHDB / participating crop protection companies for advice on selection of products and application.

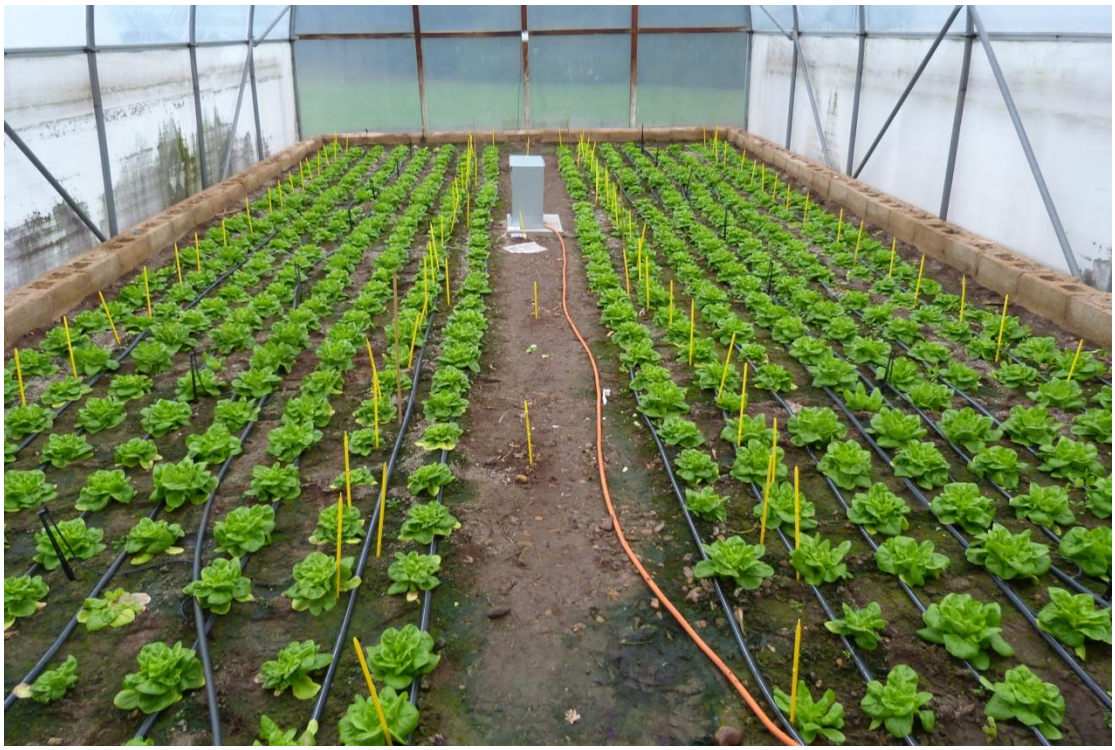
Appendix

Images of glasshouse and polytunnel trials

Part of the glasshouse trial showing effects of different treatments on Fusarium wilt of lettuce.



Severity of Fusarium wilt symptoms on lettuce plants for different treatments



Lettuce in the polytunnel trial

Lettuce with symptoms of Fusarium wilt in polytunnel trial

W2018.568: Glasshouse Trial

a. Crop diary – events related to growing crop

Crop	Cultivar	Planting/sowing date	Row width (m)
Lettuce	Temira	Sown 11/7/18, Planted 27/7/18	0.3

Previous cropping

Year	Crop
n/a	

Cultivations

Date	Description	Depth
n/a		

Active ingredient(s) / fertiliser(s) applied to the trial area

Date	Product	Rate	Unit
n/a			

Pesticides applied to the trial area

Date	Product	Rate	Unit
n/a			

Other actions

Date	Action
n/a	

b. Trial diary

Date	Event
4/7/18	Fumigant treatments applied to inoculated compost and sealed in bags
11/7/18	Seed sown
11/7/18	Sowing time treatments applied
18/7/18	Bags vented
27/7/18	Trial planted
27/7/18	Spray treatments applied

3/8/18	Spray treatments applied
3/8/18	Phytotoxicity assessed
14/8/18	Wilting leaves scored
17/8/18	Wilting leaves scored
21/8/18	Wilting leaves scored
24/8/18	Wilting leaves scored
28/8/18	Wilting leaves scored
31/8/18	Wilting leaves scored
5/9/18	Wilting leaves scored
7/9/18	Wilting leaves scored
10/9/18	Wilting leaves scored
10/9/18	Vascular browning assessed

c. Raw data from assessments

	days	18	21	25	28	32	35	40	42	45	
	date	14/8/18	17/8/18	21/8/18	24/8/18	28/8/18	31/8/18	5/9/18	7/9/18	10/9/18	Final Score
plot	**treatment**	Score (0-5 scale)									0-4 scale
1	3	1	1	1	2	3	3	3	3	4	3
1	3	1	2	2	3	3	3	3	4	4	3
1	3	1	1	1	1	2	2	3	3	3	3
2	14	1	2	2	3	4	4	4	4	5	4
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12	10	2	2	3	4	4	4	4	4	5	4
12	10	2	2	3	3	4	5	5	5	5	4
12	10	2	2	3	3	4	4	4	4	4	3
13	8	2	2	2	2	2	2	2	2	3	3
13	8	3	3	3	3	3	3	3	3	4	3
13	8	3	3	3	3	3	3	3	3	4	3
15	5	1	1	1	1	1	1	1	1	1	3
15	5	1	1	1	1	1	1	1	1	1	2
15	5	0	1	1	1	2	2	2	2	3	3
16	14	3	3	3	3	2	2	3	3	4	3
16	14	2	2	2	2	3	3	3	3	3	3
16	14	2	2	2	3	3	3	3	4	4	3

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1	13	2	2	2	2	2	2	3	3	4	3
1	13	3	3	4	5	5	5	5	5	5	4
2	1	2	2	2	2	2	2	3	3	3	3
2	1	2	2	2	2	2	2	2	2	2	3
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5	3	3	3	3	3	3	3	3	3	3	3
6	5	2	2	2	2	1	1	1	1	1	2
6	5	1	1	1	2	2	2	2	2	2	3
6	5	1	1	1	1	2	2	2	2	2	2
7	7	4	4	4	5	5	5	5	5	5	4
7	7	2	2	2	2	3	3	3	3	3	3
7	7	2	2	3	4	4	4	4	5	5	4
8	16	3	3	3	4	4	4	4	5	5	4
8	16	3	3	3	3	3	4	4	4	4	3
8	16	3	4	4	4	4	5	5	5	5	4
9	6	3	3	3	3	2	2	2	3	3	3
9	6	1	1	1	2	2	2	2	2	2	2
9	6	3	3	3	3	3	3	3	4	4	3
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11	15	0	0	0	0	0	0	0	0	0	0
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11	15	0	0	0	0	0	0	0	0	0	0
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12	11	0	1	1	1	1	1	1	2	2	3
12	11	4	4	4	5	5	5	5	5	5	4
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13	10	2	2	2	3	3	4	4	4	4	3
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16	12	3	3	4	4	4	4	4	4	5	4
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4	8	2	2	2	3	3	3	3	3	4	3
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5	12	2	2	3	4	4	4	4	4	4	3
5	12	2	2	2	3	3	4	4	4	4	3
5	12	2	2	2	2	3	4	3	4	4	3
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6	2	0	0	0	0	0	0	0	0	0	0
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7	13	1	1	1	2	3	3	3	3	3	3
7	13	2	3	3	4	4	4	4	4	5	4
8	7	2	3	4	4	4	4	4	4	5	4
8	7	4	4	4	5	5	5	5	5	5	4
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9	1	3	3	3	3	4	4	5	5	5	4
9	1	2	2	2	3	3	3	3	3	4	3
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11	14	1	1	1	2	2	2	2	2	5	4
11	14	1	1	1	2	3	3	3	4	4	3
11	14	3	3	3	4	4	4	5	5	5	4
12	9	2	2	3	3	4	4	3	5	5	4
12	9	2	2	2	2	2	2	3	3	4	3
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15	15	0	0	0	0	0	0	0	0	0	2
15	15	0	0	0	0	0	0	0	0	0	0
16	5	1	1	1	1	1	1	1	2	2	3
16	5	1	1	1	1	2	2	2	3	3	3
16	5	1	1	1	1	1	1	1	1	1	2
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1	13	2	3	4	4	4	4	4	4	4	3
1	13	4	4	4	4	5	5	5	5	5	4
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4	3	3	3	3	3	3	3	3	4	4	3
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5	16	3	3	4	4	4	4	4	5	5	4

5	16	4	4	4	4	5	5	5	5	5	4
6	6	1	1	1	2	2	2	2	2	2	3
6	6	3	3	4	4	5	5	5	5	5	4
6	6	3	4	4	5	5	5	5	5	5	4
7	11	2	2	3	4	4	4	4	5	5	4
7	11	2	3	3	4	5	5	5	5	5	4
7	11	2	2	2	3	3	3	4	4	4	3
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10	9	2	2	2	3	3	3	3	3	3	3
11	12	1	1	2	2	2	2	2	2	3	3
11	12	2	2	2	2	2	2	2	3	3	2
11	12	3	3	4	4	5	5	5	5	5	4
12	5	1	1	1	1	2	2	2	2	2	2
12	5	1	1	1	1	1	1	1	1	1	3
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13	8	3	3	4	5	5	5	5	5	5	4
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14	2	0	0	0	0	0	0	0	0	0	1
14	2	0	0	0	0	0	0	0	0	0	0
14	2	0	0	0	0	0	0	0	0	0	0
15	7	2	2	2	3	3	3	3	3	3	3
15	7	2	2	2	3	4	4	4	4	5	4
15	7	4	4	4	4	4	4	5	5	5	4
16	10	3	4	4	4	4	4	4	5	5	4
16	10	3	4	4	4	4	4	5	5	5	4
16	10	3	3	4	4	5	5	5	5	5	4
1	2	0	0	0	0	0	0	0	0	0	0
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3	8	3	3	4	5	5	5	5	5	5	4
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3	8	2	2	3	4	4	4	5	5	5	4
4	1	3	3	4	4	4	4	5	5	5	4
4	1	3	4	4	4	5	5	5	5	5	4
4	1	2	2	2	3	3	3	3	3	4	3
5	14	2	2	2	3	3	4	4	4	5	4
5	14	2	2	2	3	3	3	3	4	4	3
5	14	2	2	2	3	3	3	4	4	5	4
6	3	3	3	4	4	5	5	5	5	5	4
6	3	1	1	1	1	2	2	2	2	2	2
6	3	1	1	1	1	1	1	2	2	2	2
7	16	3	4	4	4	5	5	5	5	5	4
7	16	2	2	2	3	4	4	4	4	5	4
7	16	2	2	3	4	4	4	5	5	5	4

8	11	2	3	3	4	5	5	5	5	5	4
8	11	2	2	2	3	3	4	4	4	5	4
8	11	4	4	4	5	5	5	5	5	5	4
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9	10	3	3	3	3	4	4	4	4	4	3
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12	12	2	2	2	3	3	3	3	4	5	4
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14	6	2	2	3	3	4	4	4	4	4	3
14	6	2	2	2	2	2	2	2	2	3	3
15	5	1	1	1	2	2	2	2	2	2	3
15	5	1	1	1	1	1	1	1	1	1	1
15	5	1	1	1	1	1	1	1	2	2	3
16	15	0	0	0	0	0	0	0	0	0	0
16	15	0	0	0	0	0	0	0	0	0	1
16	15	0	0	0	0	0	0	0	0	0	1
1	7	2	2	2	3	3	3	3	4	4	3
1	7	2	2	2	2	2	2	3	3	4	3
1	7	2	2	2	2	3	3	4	4	5	4
2	15	0	0	0	0	0	0	0	0	0	0
2	15	0	0	0	0	0	0	0	0	0	0
2	15	0	0	0	0	0	0	0	0	0	0
3	14	1	1	2	3	3	3	3	4	4	3
3	14	1	1	1	3	3	3	3	4	4	3
3	14	2	3	3	4	4	4	4	5	5	4
4	12	3	3	4	4	5	5	5	5	5	4
4	12	2	2	2	2	3	3	3	3	3	3
4	12	3	2	2	3	4	4	4	4	4	3
5	6	2	2	2	2	2	2	2	2	2	3
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5	6	3	3	4	4	4	4	4	4	5	4
6	1	3	3	3	4	4	5	5	5	5	4
6	1	2	2	2	3	3	3	3	3	3	3
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7	16	2	3	4	4	4	4	4	4	4	3
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7	16	2	2	2	3	4	4	4	4	4	3
8	4	1	2	2	3	3	4	4	4	5	4
8	4	2	2	3	4	4	4	4	4	4	3
8	4	2	2	2	3	3	3	4	4	4	3
9	9	1	1	1	2	2	2	2	2	3	3
9	9	1	2	2	2	2	2	3	3	3	2
9	9	1	2	2	3	4	4	4	4	5	4
10	10	3	4	4	4	4	4	4	5	5	4

10	10	2	2	2	4	4	4	4	5	5	4
10	10	2	2	2	3	3	3	3	3	4	2
11	5	0	0	0	0	0	0	1	1	1	2
11	5	0	0	0	1	1	1	1	1	1	3
11	5	0	0	0	1	1	1	1	1	1	3
12	13	2	3	4	4	4	4	4	4	5	4
12	13	2	2	2	3	3	3	3	3	3	2
12	13	0	1	1	2	3	3	3	3	3	3
13	2	0	0	0	0	0	0	0	0	0	0
13	2	0	0	0	0	0	0	0	0	0	1
13	2	0	0	0	0	0	0	0	0	0	0
14	11	2	2	2	2	3	3	3	4	4	3
14	11	3	3	4	4	4	4	4	5	5	4
14	11	1	1	1	2	3	3	3	3	3	3
15	8	2	2	4	4	4	4	4	4	5	4
15	8	1	2	2	2	2	2	2	2	2	3
15	8	3	3	4	4	4	4	4	4	4	3
16	3	3	4	4	4	5	5	5	5	5	4
16	3	1	1	1	2	2	2	2	2	2	3
16	3	0	0	0	1	2	2	2	2	2	1

d. Design

	BENCH 1			BENCH 2		
BLOCK 1	3	14		16	4	BLOCK 5
	12	1		11	8	
	4	9		12	2	
	15	8		13	7	
	7	10		1	3	
	11	6		14	9	
	2	5		6	10	
	16	13		15	5	
BLOCK 2	13	3		13	15	BLOCK 6
	1	14		14	3	
	9	7		16	6	
	5	15		11	4	
	4	10		1	9	
	11	6		12	5	
	2	16		8	2	
	12	8		7	10	
BLOCK 3	4	2		2	13	BLOCK 7
	9	16		8	1	
	7	13		14	3	
	6	1		16	11	
	15	11		10	7	
	12	10		4	12	
	8	3		9	6	
	5	14		5	15	
BLOCK 4	13	1		7	15	BLOCK 8
	14	9		14	12	
	3	5		6	1	
	7	16		16	4	
	6	8		9	10	
	15	11		5	13	
	10	2		2	11	
	4	12		8	3	

W2018.569: Polytunnel Trial

a. Crop diary – events related to growing crop

Crop	Cultivar	Planting/sowing date	Row width (m)
Lettuce	Tirmira	Sown 27/9/18, Planted 11/10/18	0.3

Previous cropping

Year	Crop
2018	Lettuce

Cultivations

Date	Description	Depth
19/9/18	Rotavation	15 cm

Active ingredient(s) / fertiliser(s) applied to the trial area

Date	Product	Rate	Unit
19/9/18	P	150	Kg/ha
19/9/18	K	150	Kg/ha
19/9/18	Nitram	300	Kg N/ha

Pesticides applied to the trial area

Date	Product	Rate	Unit
7/12/18	Signum	1.5	kg/ha
21/12/18	Signum	1.5	kg/ha

Other actions

Date	Action
15/11/18	Propane heater installed

b. Trial diary

Date	Event
19/9/18	Polytunnel rotavated
20/9/18	Plots marked and Basmid treatment applied
27/9/18	Seed sown
27/9/18	T34 applied to peat blocks
11/10/18	Trial planted
11/10/18	Spray treatments applied
18/10/18	Spray treatments applied

18/10/18	Plant death assessed
19/12/18	Yellowing leaves assessed
11/1/19	Vascular browning assessed

c. Raw data from assessments

		Date	19/12/2018			11/01/2019	
Plot	Treatment	Plant	Number of yellow leaves	Botrytis	Stunting	Score (0 - 4)	Botrytis
1	1	1	4	0	0	1	0
		2	0	0	0	2	0
		3	1	0	0	3	0
		4	0	0	0	3	0
		5	5	1	1	3	0
		6	1	0	0	2	0
		7	3	0	0	3	0
		8	3	0	1	3	0
		9	2	0	0	3	0
		10	2	0	0	3	0
		11	0	0	0	3	0
		12	1	0	0	3	0
2	2	1	0	0	0	1	0
		2	3	0	0	3	0
		3	1	0	0	2	0
		4	4	0	0	3	0
		5	4	0	0	3	0
		6	2	0	1	3	0
		7	0	0	0	2	0
		8	1	0	0	1	0
		9	3	0	0		1
		10	2	0	0	3	0
		11	5	1	1	2	0
		12	2	0	0		1
3	3	1	0	0	0	1	0
		2	0	0	0	3	0
		3	1	0	0	2	0
		4	1	0	0	2	0
		5	0	0	0	2	0
		6	4	0	1		1
		7	0	0	0	1	0
		8	4	0	0	3	0
		9	2	0	0	3	0
		10	3	0	0	3	1
		11	3	0	0	2	0
		12	1	0	1	2	0
4	4	1	0	0	0	0	0

		2	0	0	0	0	0
		3	1	1	0		1
		4	0	0	0	0	0
		5	1	0	0	0	0
		6	0	0	0		0
		7	1	0	0	0	0
		8	0	0	0	0	0
		9	dead	1	0		1
		10	0	0	0	0	0
		11	0	0	0	0	0
		12	0	0	0	0	0
5	5	1	0	0	0	2	0
		2	1	0	0	3	0
		3	0	0	0	2	0
		4	5	0	1	3	1
		5	1	0	0	3	0
		6	3	1	1	3	0
		7	0	0	0	2	0
		8	2	0	0	2	0
		9	2	0	0	2	0
		10	1	0	0	2	0
		11	1	0	0	2	0
		12	3	0	0	2	0
6	1	1	0	0	0	0	0
		2	0	0	0	2	0
		3	1	0	0	2	0
		4	0	0	0	2	0
		5	4	1	0	3	0
		6	2	0	0	2	0
		7	1	0	0	3	0
		8	0	0	0	2	0
		9	1	0	0	2	0
		10	2	0	0	2	0
		11	0	0	0	2	0
		12	1	0	0	1	0
7	3	1	0	0	0	2	0
		2	1	0	0	3	0
		3	0	0	0	2	0
		4	0	0	0	1	0
		5	0	0	0	1	0
		6	2	0	0	2	0
		7	2	0	0	2	0
		8	0	0	0	2	0
		9	0	0	0	2	0
		10	0	0	0	2	0
		11	2	0	0	1	0
		12	0	0	0	2	0

8	4	1	dead	1	0		0
		2	0	0	0	0	0
		3	0	0	0	0	0
		4	0	0	0	0	0
		5	0	0	0	0	0
		6	0	0	0	0	0
		7	0	0	0	0	0
		8	0	0	0	0	0
		9	0	0	1		0
		10	0	0	0	0	0
		11	0	0	0	0	0
		12	0	0	1	0	0
9	2	1	1	1	0	1	1
		2	0	0	0	2	0
		3	1	0	0	2	0
		4	3	0	0	2	0
		5	4	0	0	3	0
		6	7	0	0	2	0
		7	2	0	0	2	0
		8	3	0	0	3	0
		9	3	0	1	3	0
		10	0	0	0	2	0
		11	0	0	0	2	0
		12	2	0	1	1	0
10	5	1	1	0	0	2	0
		2	1	0	0	2	0
		3	0	0	0	2	0
		4	3	1	1		0
		5	0	0	0	1	0
		6	4	0	1	1	0
		7	0	0	0	3	0
		8	1	0	0	2	0
		9	5	0	0	3	0
		10	3	1	1		0
		11	3	0	0	2	0
		12	2	0	0	2	0
11	2	1	4	1	0	2	0
		2	0	0	0	3	0
		3	1	0	0	2	0
		4	5	1	1		0
		5	6	1	1	2	0
		6	2	0	0	2	0
		7	4	0	0	2	0
		8	3	0	0	2	0
		9	5	1	0	2	0
		10	2	0	0	2	0
		11	3	0	1	3	0
		12	1	0	0	0	0
12	5	1	5	1	0	2	1
		2	2	0	0	2	0
		3	0	0	0	1	0
		4	1	0	0	3	0
		5	4	0	0	3	0

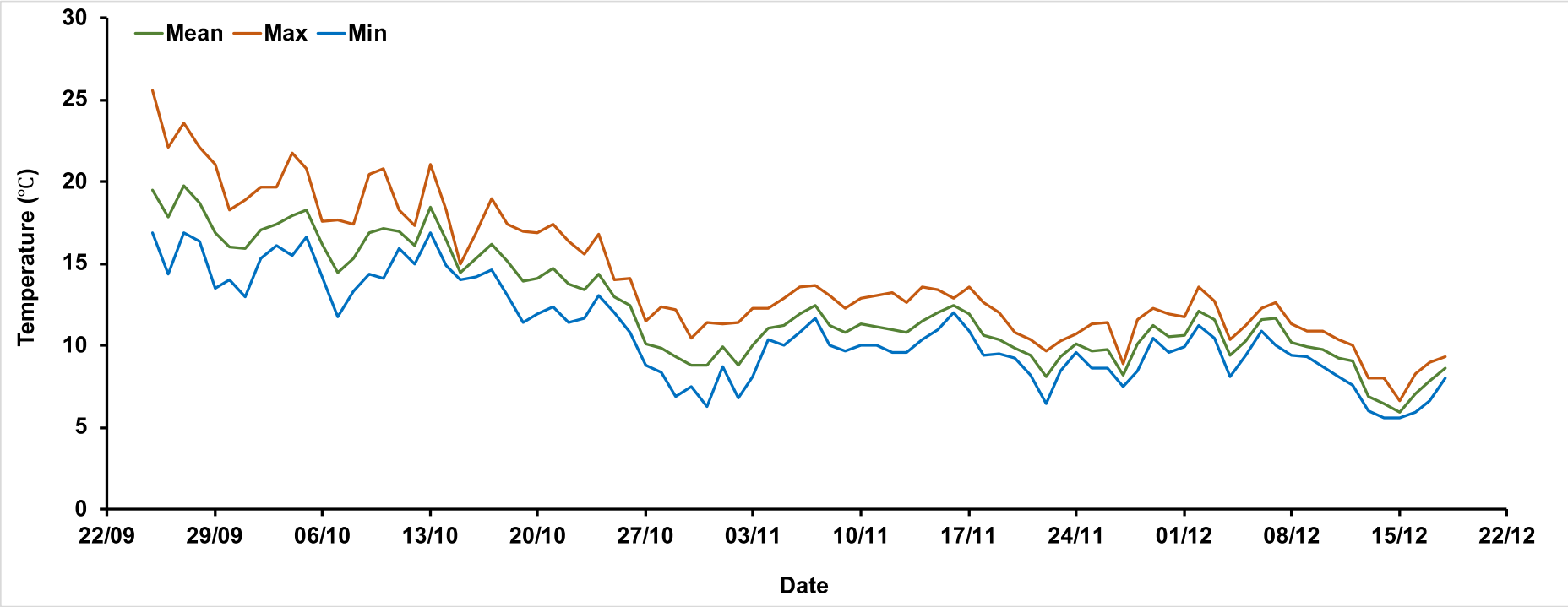
		6	4	0	1	3	0
		7	3	0	0	2	0
		8	2	0	1	3	0
		9	1	0	0	2	0
		10	5	1	0	3	1
		11	5	0	0	2	0
		12	3	0	0	1	0
13	4	1	0	0	0	0	0
		2	0	0	0	0	0
		3	0	0	0	0	0
		4	0	0	0	0	0
		5	1	0	0	0	0
		6	0	0	0	0	0
		7	1	0	0		1
		8	0	0	0	0	0
		9	0	0	0	0	0
		10	0	0	0	0	0
		11	0	0	0	0	0
		12	0	0	0	0	0
14	3	1	0	0	0	2	0
		2	dead	1	0		0
		3	0	0	0	2	0
		4	0	0	0	1	0
		5	0	0	0	1	0
		6	2	0	0	1	0
		7	0	0	0	1	0
		8	3	1	0		1
		9	1	0	0	2	0
		10	1	1	0	1	0
		11	1	0	0	1	0
		12	2	1		2	0
15	1	1	3	0	0	2	0
		2	3	0	0	2	0
		3	2	0	0	2	0
		4	1	0	0	2	0
		5	4	0	0	1	0
		6	2	0	0	2	0
		7	3	0	0	2	1
		8	2	0	0	3	0
		9	2	0	0	3	0
		10	2	0	0	2	0
		11	1	0	0	1	0
		12	3	1	1		1
16	2	1	0	0	0	1	0
		2	1	0	0	1	0
		3	0	0	0	2	0
		4	0	0	0	0	0
		5	3	1	0		1
		6	1	0	0	1	0
		7	0	0	0	2	0
		8	3	0	0	2	0
		9	0	0	0	1	0
		10	1	1	0	1	0
		11	0	0	0	2	0
		12	0	0	0	1	0

17	5	1	2	0	1	3	0
		2	2	1	1	2	0
		3	1	0	0	1	0
		4	0	0	0	0	0
		5	dead	1	1		0
		6	0	0	0	1	0
		7	0	0	0	2	0
		8	1	0	0	1	0
		9	0	0	0	2	0
		10	0	0	0	1	0
		11	0	0	0	1	0
		12	1	0	0	1	0
18	1	1	4	0	0	2	0
		2	0	0	0	2	0
		3	0	0	0	0	0
		4	1	0	0	1	0
		5	0	0	0	1	0
		6	0	0	0	2	0
		7	3	0	0	2	0
		8	5	1	1	2	0
		9	0	0	0	1	0
		10	0	0	0	0	0
		11	0	0	0	2	0
		12	2	0	0	2	0
19	4	1	0	0	1	0	0
		2	dead	1	0		0
		3	0	0	0	0	0
		4	0	0	0	0	0
		5	dead	0	0		0
		6	0	0	0	0	0
		7	0	0	0	0	0
		8	0	0	0	0	0
		9	0	0	0	0	0
		10	1	0	0	0	0
		11	0	0	0	0	0
		12	0	0	0	0	0
20	3	1	0	0	0	2	0
		2	1	0	0	2	0
		3	1	0	0	2	0
		4	3	0	0	2	0
		5	0	0	0	0	0
		6	0	0	0	0	0
		7	0	0	0	2	0
		8	2	0	0	2	0
		9	1	0	0	0	0
		10	0	0	0	0	0
		11	0	0	0	2	0
		12	1	0	0	2	0

d. Design

BLOCK 1	1 2 3 4 5		2 5 4 3 1	BLOCK 3
BLOCK 2	1 3 4 2 5		2 5 1 4 3	BLOCK 4

e. Temperature data from polytunnel trial



Certificate of

Official Recognition of Efficacy Testing Facilities or Organisations in the United Kingdom

This certifies that

Warwick Crop Centre, School of Life Sciences

complies with the minimum standards laid down in Regulation (EC) 1107/2009 for efficacy testing.

The above Facility/Organisation has been officially recognised as being competent to carry out efficacy trials/tests in the United Kingdom in the following categories:

Agriculture/Horticulture
Biologicals and Semiochemicals

Date of issue: **6 October 2017**
Effective date: **20 March 2017**
Expiry date: **19 March 2022**

Signature Alison Richardson
Authorised signatory

Certification Number
ORETO 381

HSE
Chemicals Regulation Division

Department of
Agriculture and
Rural Development