SCEPTREPLUS

Final Trial Report

Trial code:	W2018.568 (Glasshouse) and W2018.569 (Polytunnel)
Title:	SP25a: 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:	April 2021 – November 2021
Report date:	
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.

28/01/2022	John Clarkson
Date	**Authors signature**

Trial Summary

Introduction

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

Polytunnel trial

Method: A quarantine polytunnel previously inoculated with FOL4 was used to test four microbial biofungicide treatments for their ability to reduce Fusarium wilt in lettuce; T34 Biocontrol, Trianum G / Trianum P, Prestop and Serifel. Disease pressure was anticipated to be low-moderate as only one lettuce crop had been grown in the area since inoculation and the original plan was to examine the effect of products over two sequential lettuce crops. Lettuce plants (cv. Amica) were raised in peat blocks and transplanted into the polytunnel soil on 27/05/21 in plots each containing 14 lettuce plants at 30 cm spacing. There were four replicate plots for each treatment arranged in a randomised block design. Treatments were applied to peat blocks at sowing and with a knapsack to plants at transplanting and 7 days later. Fusarium wilt symptoms were assessed each week. In addition, levels of FOL4 in the soil were quantified by a specific qPCR assay before transplanting and after the experiment was terminated (plants still present).

Results: Fusarium wilt symptoms developed very rapidly for all treatments and >80% of plants died within two weeks (results not shown). This trial was therefore terminated mid-wat through crop 1 due to excessive disease pressure and replaced with a glasshouse trial. FOL4 DNA levels declined between pre-trial and end-of-trial samples across all treatments as well as the untreated control; there was therefore no evidence that any of the treatments reduced FOL4 inoculum levels.

Glasshouse trial

Method: Four microbial biofungicide treatments T34 Biocontrol, Trianum G / Trianum P, Prestop and Serifel were tested for their ability to reduce Fusarium wilt in lettuce in inoculated pot trials in the glasshouse. Lettuce plants (cv. Amica) were raised in peat blocks for 16 days after which they were transplanted into in 9 cm square pots containing compost infested with FOL4 at two different levels (1×10^5 cfu g^{-1} - moderate and 1×10^6 cfu g^{-1} - high). There was a total of 20 pots per treatment which were placed into a glasshouse compartment in a randomised block design with set points of 25°C day / 18°C night. Treatments were applied to peat blocks at sowing using a pipette and with a knapsack to plants at transplanting and 7 days later (with the exception of Trianum). Fusarium wilt symptoms were assessed each week and scored between 0 (healthy) and 5 (dead). At the end of the trial, plants were dissected and vascular browning due to Fusarium assessed using a score between 0 (healthy) and 4 (severe vascular browning).

Results: Fusarium wilt disease developed at both moderate and high levels of FOL4 inoculum with final wilt scores of 2.3 and 4.9 and vascular browning scores of 1.5 and 3.9 for the untreated control treatments for moderate and high levels of FOL4 inoculum respectively. Disease score was relatively low for the untreated control with the moderate level of inoculum and none of the treatments resulted in a decrease in Fusarium wilt, with some causing a significant increase in disease (Table 1). At the high level of FOL4 inoculum, none of the treatments resulted in consistent reduction in Fusarium wilt, although Trianum and Serifel

resulted in a significant reduction in disease at single timepoints. No treatment significantly reduced vascular browning due to Fusarium at harvest.

Table 1: Effect of microbial biofungicides on mean Fusarium wilt and vascular browning symptom scores in lettuce grown in compost infested with different levels of FOL4 inoculum in the glasshouse trial.

			Wilting Score						VB Score
Treat. No.	**Product**	**FOL4 inoculum level**	**27-Oct**	**03-Nov**	**10-Nov**	**17-Nov**	**24-Nov**	**01-Dec**	**02-Dec**
1	Untreated	None	0.00	0.00	0.00	0.00	0.05	0.05	0.00
2	Untreated	Moderate	0.00	0.35	0.95	1.45	1.95	2.30	1.50
3	T34	Moderate	0.00	0.55	0.90	1.70	2.35	2.65	2.10
4	Prestop	Moderate	0.00	0.60	1.10	1.85	2.10	2.50	2.05
5	Trianum	Moderate	0.00	0.50	1.50	2.10	2.50	3.10	2.45
6	Serifel	Moderate	0.00	0.60	1.40	2.05	2.50	2.90	2.45
7	Untreated	High	0.30	1.65	2.50	3.60	4.35	4.85	3.90
8	T34	High	0.15	1.55	2.20	3.40	4.20	4.80	4.00
9	Prestop	High	0.30	1.60	2.60	3.60	4.30	4.95	4.00
10	Trianum	High	0.25	1.55	2.30	3.15	4.05	4.80	3.85
11	Serifel	High	0.25	1.25	2.15	3.20	3.80	4.65	3.70
		F	3.79	16.46	26.53	57.52	71.40	94.49	69.97
		P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
		SED	0.099	0.209	0.225	0.210	0.226	0.226	0.219
		LSD (5%)	0.195	0.412	0.444	0.415	0.445	0.446	0.432
		df	209	209	209	209	209	209	209

Significantly lower than untreated control at the same level of inoculum
Significantly greater than inoculated control at the same level of inoculum

Conclusions

- Application of the microbial biofungicides tested did not result in any consistent reduction in lettuce Fusarium wilt in an inoculated trial in the glasshouse.
- Treatments did not reduce the level of FOL4 inoculum in the polytunnel soil, as measured by qPCR.
- Results contrast with a trial in 2018 where T34 Biocontrol significantly reduced Fusarium wilt in a similar pot-based trial.
- Other approaches need to be developed for Fusarium wilt control; this could include resistant lettuce cultivars, soil sterilisation or use of microbial biofungicides to prevent build-up of FOL4 inoculum.

Take home message: The microbial biofungicides tested had little or no activity against Fusarium wilt in artificially inoculated pot trials.

Objectives

1. To evaluate the effectiveness of biofungicides for the control of Fusarium wilt caused by FOL4 in a polytunnel trial and quantify pathogen levels using qPCR.
2. To evaluate the effectiveness of biofungicides for the control of Fusarium wilt caused by FOL4 in a glasshouse pot trial.

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.

Treatment details (Polytunnel and Glasshouse trials)

AHDB Code	Active substance	Product name/ manufacturer code	Formulation batch number	Content of active substance in product	Formulation type[1]	Adjuvant
	Untreated, Inoculated control					
Approved	*Trichoderma asperellum* strain T34	T34	09192018	1×10^{12} cfu g^{-1}	WP	None
Approved	*Trichoderma harzianum* strain T22	Trianum G	20TG45	1.5×10^{8} cfu g^{-1}	GR	None
Approved	*Trichoderma harzianum* strain T22	Trianum P	20TP29	1×10^{9} cfu g^{-1}	WP	None
Approved	*Gliocladium catenulatum* strain JI J1446	Prestop	SAL2564389	2×10^{8} cfu g^{-1}	WP	None
Approved	*Bacillus amyloquefaciens* strain MBI 600	Serifel	0017453567	5.5×10^{10} cfu g^{-1}	WP	None

[1] WP, wettable powder; GR, granule

Polytunnel Trial

Test site

Item	Details
Location address	University of Warwick Wellesbourne Campus Wellesbourne Warwick CV35 9EF
Crop	Lettuce
Cultivar	Amica
Soil or substrate type	Potgrond P (Klas) – blocking compost Sandy loam soil - polytunnel
Agronomic practice	N/A
Prior history of site	N/A

Trial design

Item	Details
Trial design:	Incomplete Latin square
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:	14

Methods, assessments and records

A quarantine polytunnel previously inoculated with FOL4 was used to test four microbial biofungicide treatments for their ability to reduce Fusarium wilt in lettuce; T34 Biocontrol, Trianum G / Trianum P, Prestop and Serifel. Disease pressure was anticipated to be low-moderate as only one lettuce crop had been grown in the area since inoculation and the original plan was to examine the effect of products over two sequential lettuce crops. Untreated pelleted lettuce seed (cv. Amica, butterhead type) was sown into peat blocks (4.5 x 4.5 x 4.5 cm) on 23/6/21. T34 Biocontrol, Trianum G, Prestop and Serifel treatments were mixed into a volume of compost (sufficient to make 250 blocks) before blocking (Application A) using a compost mixer. After 20 days (13/07/21), lettuce were transplanted into the polytunnel plots in plots each containing 14 lettuce plants at 30 cm spacing. There were four replicate plots for each treatment arranged in a randomised block design. Further treatments of each biofungicide (except that Trianum P was used instead of Trianum G) were applied 1 day after transplanting (14/07/21) using a knapsack sprayer fitted with a 05F110 nozzle (Application B). After 13 days (26/07/21) a second spray application of each biofungicide (excluding Trianum P) was applied (Application C). Untreated control treatments were also set up in the polytunnel and were left unsprayed.

Disease assessment details

After disease symptoms started to occur, lettuce plants were first assessed for severity of Fusarium disease on 12/08/21 by counting the number of yellowing/ wilting leaves per plant and assigning a disease score between 0 and 4 as follows: 0, healthy; 1, <10% leaves wilted; 2, 10-50% leaves wilted, 3, >50% leaves wilted; 4, 100% leaves wilted, 5, plant dead. The plants were further assessed at weekly intervals until 31/8/21.

Application schedule

Treat No.	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			
2	T34	A: 2.5 x 10^{11} cfu/250 blocks	A: 0.25 g/250 blocks BC: 5 kg/ha	ABC
3	Trianum G	A: 2.6 x 10^{9} cfu/250 blocks	A: 17.1 g/250 blocks	A
3	Trianum P	B: 1.5 x 10^{14} cfu/ha	B: 15 kg/ha	B
4	Prestop	A: 4 x 10^{9} cfu/250 blocks BC: 1.2 x 10^{12} cfu/ha	A: 20 g/250 blocks BC: 6 kg/ha	ABC
5	Serifel	A: 1.4 x 10^{9} cfu/250 blocks BC: 2.8 x 10^{13} cfu/ha	A: 0.025 g/250 blocks BC: 0.5 kg/ha	ABC

Application details

	Application A	Application B	Application C
Application date	23/06/21	14/07/21	26/07/21
Time of day	13.00	12.00	11.00
Crop growth stage (Max, min average BBCH)	N/A	14	33
Crop height (cm)	N/A	5	8
Crop coverage (%)	N/A	10	50
Application Method	Compost mixing	Spray	
Application Placement	Block	Soil/block	
Application equipment	St Moritz 300 L compost mixer	Berthoud Vermorel 2000HP	
Nozzle pressure	N/A	2 bar	
Nozzle type	N/A	05F110	
Nozzle size	N/A	05	
Application water volume	N/A	1000 l/ha[1]	
Temperature of air - shade (°C)	18	>20	>20
Relative humidity (%)	Not recorded	Not recorded	Not recorded
Wind speed range (m/s)	N/A	N/A	N/A
Dew presence (Y/N)	N/A	N/A	N/A
Temperature of soil - 2-5 cm (°C)	N/A	Not recorded	Not recorded
Wetness of soil - 2-5 cm	N/A	Not recorded	Not recorded
Cloud cover (%)	N/A	N/A	N/A

[1] Spray calibration at 30cm swath width. Spray applied at 10 cm swath width

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 wilt	*Fusarium oxysporum* f.sp. *lactucae* race 4 (FOL4)	FUSALC	0% incidence	100% incidence in untreated control; wilting score 2.6	100% incidence in untreated control; wilting score 4.9

Assessments

	Evaluation Timing (DA)*				
Evaluation date	**After sowing**	**After sprays**	**Crop Growth Stage (BBCH)**	**Evaluation type (efficacy, phytotox)**	**Assessment**
07/07/21	14	-	13	Phytotoxicity	Plant death
12/08/21	46	17	35	Efficacy	Wilting leaf score
18/08/21	52	23	35	Efficacy	Wilting leaf score
24/08/21	58	29	35	Efficacy	Wilting leaf score
31/08/21	65	36	35	Efficacy	Wilting leaf score

* DA – days after sowing (first application) and first spray application (transplanting)

Statistical analysis

This trial was designed as an incomplete Latin square for 5 treatments in a 4x5 design and wilting leaf scores (4 assessments) analysed by ANOVA.

Quantification of FOL4 inoculum levels using qPCR

Soil samples were collected before lettuce transplanting in the polytunnel on 27/05/21 and again after the experiment was terminated (01/09/21; plants still present). Small soil samples (5 x approx. 70g) were collected from each plot across each experimental block and pooled into one sample per plot. Soil was then air-dried and sieved (4 mm and 2 mm mesh) before DNA was extracted using the GeneAll Exgene Soil SV kit (Cambio, Cambridge, UK) following the manufacturers protocol, with the following modifications: 1) 550 µL of SL buffer and 200µl of SDW was added to 500 mg of soil, which was homogenised in a Powerbead tube using a FastPrep-24 (MP Biomedicals, Cambridge, UK) machine set at 5.5 m s^{-1} for three cycles of 25 seconds; 2) spin columns were incubated for 5 min after the addition of buffer EB before elution. Three triplicate DNA extractions were conducted per soil sample. Following extraction, DNA was diluted 1:2 with TE Buffer and used for qPCR analysis with FOL4 specific primers g23490 F3/R (AHDB project FV/PE 458). qPCR was carried out using a QuantStudio 5 (384-well) machine (Applied Biosystems) using 20 µL reactions containing both primers (final concentration 0.4 µM), 10 µl Power SYBR™ Green PCR Master Mix (Applied Biosystems) and 1 µl of DNA. Conditions were as follows: 1 cycle of 95°C for 120s followed by 45 cycles of 95°C for 3 s, and 60°C for 30 s. All samples were run in triplicate and a melt curve analysis carried out. The concentration of FOL4 DNA in each sample was calculated as pg DNA mg^{-1} of dry soil.

Polytunnel Trial Results

There was no evidence of phytotoxicity after block treatment or spraying (data not shown). Fusarium wilt symptoms developed very rapidly for all treatments with very high disease scores within two weeks of the first assessment (Table 1). Due to excessive disease pressure, there were no treatment differences, and it was decided to replace this approach with a glasshouse trial.

Table 2: Effect of microbial biofungicides on mean Fusarium wilt scores in lettuce grown in soil infested with FOL4 inoculum in the polytunnel trial.

Treatment no.	Product	12-Aug	18-Aug	24-Aug	31-Aug
Untreated	Untreated	2.625	3.321	4.321	4.875
T34	T34	2.179	2.661	3.893	4.679
Trianum	Trianum	2.518	2.982	4.214	4.839
Prestop	Prestop	2.321	2.714	3.857	4.696
Serifel	Serifel	2.089	2.768	3.911	4.643
F	F	1.544	1.059	0.732	0.729
P	P	0.240	0.410	0.584	0.586
SED	SED	0.256	0.372	0.352	0.172
LSD (5%)	LSD (5%)	0.545	0.793	0.750	0.366
df	df	15	15	15	15

Quantification of FOL4 inoculum levels using qPCR

Generally, levels of FOL4 DNA were lower in the soil samples collected at the end of the trial (plants still present), compared to before transplanting for all treatments including the untreated control (Figure 1). There was considerable variation in levels of FOL4 DNA detected across plots in the polytunnel, which made it more difficult to determine if any of the treatments had an effect on reducing inoculum concentration. However, for some individual plots for Serifel and T34 treatments, the levels of FOL4 were similar pre-transplanting and at end-of-trial, whereas all other replicate plots had significantly less FOL4 DNA detected in end-of-trial samples compared to the earlier samples (data not shown). Overall, there was therefore no evidence that any of the treatments reduced FOL4 inoculum levels in the soil.

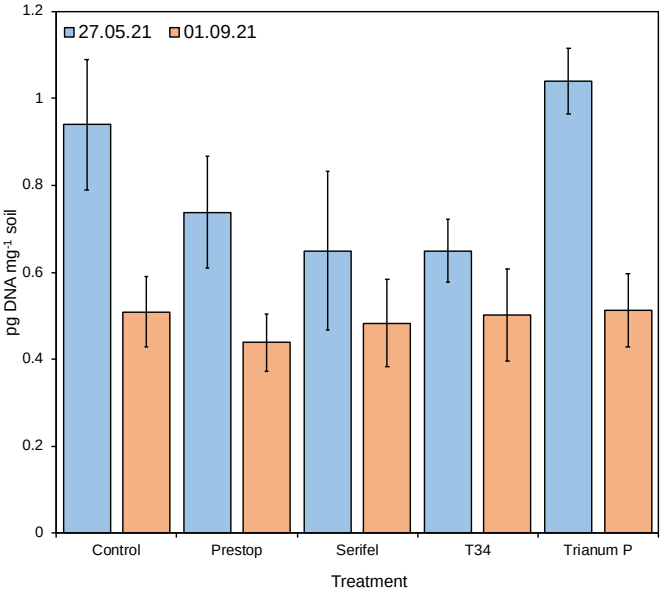

Figure 1. Amount of FOL4 DNA detected (pg DNA mg^{-1} dry soil) from polytunnel soil samples before lettuce transplanting (27.05.21) and at harvest (01.09.21). Values are means of four replicate plots per treatment. Error bars represent standard error of the mean.

Glasshouse Trial

Test site

Item	Details
Location address	University of Warwick Wellesbourne Campus Wellesbourne Warwick CV35 9EF
Crop	Lettuce
Cultivar	Amica
Soil or substrate type	Potgrond P (Klas) – blocking compost M2 compost – potting compost
Agronomic practice	N/A
Prior history of site	N/A

Trial design

Item	Details
Trial design:	Randomised block
Number of replicates:	20 replicate 9 cm pots
Row spacing:	N/A
Plot size: (w x l)	N/A
Plot size: (m^2)	N/A
Number of plants per plot:	N/A

Methods, assessments and records

Four microbial biofungicide treatments (T34 Biocontrol, Trianum G / Trianum P, Prestop and Serifel) were tested for their ability to reduce lettuce Fusarium wilt in an artificially inoculated pot-based glasshouse trial. Untreated pelleted lettuce seed (cv. Amica butterhead type) was sown into peat blocks (4.5 x 4.5 x 4.5 cm) on /21. T34 Biocontrol, Trianum G, Prestop and Serifel treatments were mixed into a volume of compost (sufficient to make 250 blocks) before blocking (Application A) using a compost mixer. After 22 days, lettuce were transplanted into pots containing compost inoculated with FOL4 at two different levels; 1 x 10^5 cfu g^{-1} (moderate) and 1 x 10^6 cfu g^{-1} (high) in square 9 cm pots. There were 20 replicate plots for each treatment arranged in a randomised block design in a glasshouse compartment with conditions set at 25°C day / 18°C night with a 16 hour day length. Further treatments of each biofungicide (except that Trianum P was used instead of Trianum G) were applied immediately after transplanting on 13/10/21 using a knapsack sprayer fitted with a 05F110 nozzle (Application B). On 20/10/21, 7 days after transplanting a second spray application of each biofungicide (excluding Trianum P) was applied (Application C). Untreated control treatments were also set up in the polytunnel and were left unsprayed.

Disease assessment details

After disease symptoms started to occur, lettuce plants were first assessed for severity of Fusarium disease on 27/10/21 by counting the number of yellowing/ wilting leaves per plant and assigning a disease score between 0 and 4 as follows: 0, healthy; 1, <10% leaves wilted; 2, 10-50% leaves wilted, 3, >50% leaves wilted; 4, 100% leaves wilted, 5, plant dead. The plants were further assessed at weekly intervals until 1/12/21. At the end of the experiment (02/12/21), individual lettuce heads were cut in half longitudinally through the root and scored for severity of internal vascular browning using the following scoring system: 0, healthy; 1, mild

vascular browning; 2, moderate vascular browning; 3, severe vascular browning; 4, dead / total browning.

Application schedule

Treat No.	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			
2	T34	A: 2.5 x 10^{11} cfu/250 blocks	A: 0.25 g/250 blocks BC: 5 kg/ha	ABC
3	Trianum G	A: 2.6 x 10^{9} cfu/250 blocks	A: 17.1 g/250 blocks	A
3	Trianum P	B: 1.5 x 10^{14} cfu/ha	B: 15 kg/ha	B
4	Prestop	A: 4 x 10^{9} cfu/250 blocks BC: 1.2 x 10^{12} cfu/ha	A: 20 g/250 blocks BC: 6 kg/ha	ABC
5	Serifel	A: 1.4 x 10^{9} cfu/250 blocks BC: 2.8 x 10^{13} cfu/ha	A: 0.025 g/250 blocks BC: 0.5 kg/ha	ABC

Application details

	Application A	Application B	Application C
Application date	12/9/21	13/10/21	20/10/21
Time of day	11.00	13.00	11.00
Crop growth stage (Max, min average BBCH)	N/A	14	33
Crop height (cm)	N/A	5	8
Crop coverage (%)	N/A	10	50
Application Method	Compost mixing	Spray	
Application Placement	Block	Soil/block	
Application equipment	St Moritz 300 L compost mixer	Berthoud Vermorel 2000HP	
Nozzle pressure	N/A	2 bar	
Nozzle type	N/A	05F110	
Nozzle size	N/A	05	
Application water volume	N/A	1000 l/ha[1]	
Temperature of air - shade (°C)	18	15	14
Relative humidity (%)	Not recorded	Not recorded	Not recorded
Wind speed range (m/s)	N/A	N/A	N/A
Dew presence (Y/N)	N/A	N/A	N/A
Temperature of soil - 2-5 cm (°C)	N/A	Not recorded	Not recorded
Wetness of soil - 2-5 cm	N/A	Not recorded	Not recorded
Cloud cover (%)	N/A	N/A	N/A

[1] Spray calibration at 30cm swath width. Spray applied at 10 cm swath width

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 wilt	*Fusarium oxysporum* f.sp. *lactucae* race 4 (FOL4)	FUSALC	0% incidence	Mean wilting score 0.0 and 0.3 for moderate / high inoculum levels	Mean wilting score 1.5 and 3.9 for moderate / high inoculum levels

Assessments

	Evaluation Timing (DA)*				
Evaluation date	**After sowing**	**After sprays**	**Crop Growth Stage (BBCH)**	**Evaluation type (efficacy, phytotox)**	**Assessment**
05/10/21	14		13	Phytotoxicity	Plant death
27/10/21	36	14	33	Efficacy	Wilting leaf score
03/11/21	43	21	35	Efficacy	Wilting leaf score
10/11/21	50	28	35	Efficacy	Wilting leaf score
17/11/21	57	35	37	Efficacy	Wilting leaf score
24/11/21	64	42	37	Efficacy	Wilting leaf score
01/12/21	71	49	39	Efficacy	Wilting leaf score
02/12/21	72	56	39	Efficacy	Vascular browning score

* DA – days after sowing (first application) and first spray application (transplanting)

Statistical analysis

This trial was designed as an incomplete Latin square for 5 treatments in a 4x5 design. The wilting leaf scores (6 assessments) and vascular browning scores at harvest were analysed by ANOVA.

Glasshouse Trial Results

There was no evidence of phytotoxicity after block treatment or spraying (data not shown). Fusarium wilt disease developed at both moderate and high levels of FOL4 inoculum with final wilt scores of 2.3 and 4.9 and vascular browning scores of 1.5 and 3.9 for the untreated control treatments for moderate and high levels of FOL4 inoculum respectively. Disease score was relatively low for the untreated control with the moderate level of inoculum and none of the treatments resulted in a decrease in Fusarium wilt, with some causing a significant increase in disease (Table 3). At the high level of FOL4 inoculum, none of the treatments resulted in consistent reduction in Fusarium wilt, although Trianum and Serifel resulted in a significant reduction in disease at single timepoints. No treatment significantly reduced vascular browning due to Fusarium at harvest.

Table 3: Effect of microbial biofungicides on mean Fusarium wilt and vascular browning symptom scores in lettuce grown in compost infested with different levels of FOL4 inoculum in the glasshouse trial.

			Wilting Score						VB Score
Treat. No.	**Product**	**FOL4 inoculum level**	**27-Oct**	**03-Nov**	**10-Nov**	**17-Nov**	**24-Nov**	**01-Dec**	**02-Dec**
1	Untreated	None	0.00	0.00	0.00	0.00	0.05	0.05	0.00
2	Untreated	Moderate	0.00	0.35	0.95	1.45	1.95	2.30	1.50
3	T34	Moderate	0.00	0.55	0.90	1.70	2.35	2.65	2.10
4	Prestop	Moderate	0.00	0.60	1.10	1.85	2.10	2.50	2.05
5	Trianum	Moderate	0.00	0.50	1.50	2.10	2.50	3.10	2.45
6	Serifel	Moderate	0.00	0.60	1.40	2.05	2.50	2.90	2.45
7	Untreated	High	0.30	1.65	2.50	3.60	4.35	4.85	3.90
8	T34	High	0.15	1.55	2.20	3.40	4.20	4.80	4.00
9	Prestop	High	0.30	1.60	2.60	3.60	4.30	4.95	4.00
10	Trianum	High	0.25	1.55	2.30	3.15	4.05	4.80	3.85
11	Serifel	High	0.25	1.25	2.15	3.20	3.80	4.65	3.70
		F	3.79	16.46	26.53	57.52	71.40	94.49	69.97
		P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
		SED	0.099	0.209	0.225	0.210	0.226	0.226	0.219
		LSD (5%)	0.195	0.412	0.444	0.415	0.445	0.446	0.432
		df	209	209	209	209	209	209	209

Significantly lower than untreated control at the same level of inoculum
Significantly greater than inoculated control at the same level of inoculum

Discussion

Overall, none of the microbial biofungicide treatments reduced Fusarium wilt in either the polytunnel or glasshouse trials. This was despite careful attention to application and product rates in discussion with manufacturers, an approach to try and establish the biological control agents on lettuce roots and in modules during propagation and 'topping up' populations after planting. This is in contrast to a previous glasshouse pot trial (SP25) where T34 Biocontrol significantly reduced Fusarium disease following the same application approach. However, in related work in an MSc summer project at Warwick (2021), high doses (x 10 recommended rate) of T34 and Trianum-G were effective in reducing the severity of Fusarium wilt in lettuce in pot trials carried out in controlled environment (25C) when applied during propagation and at planting. Moreover, T34 and Trianum-G (x 10 recommended rate) provided effective control of Fusarium when lettuce seeds were sown into compost in which both the products and FOL4 had been incubated for 14 days. This therefore suggests that applications of these microbial biofungicides at higher than recommended application rates may be beneficial for control of Fusarium wilt.

A major factor in the potential success of microbial biofungicide treatments for control of Fusarium wilt is the level of FOL4 inoculum and it is clear that the pathogen is challenging to control under high disease pressure situations. Despite using two different concentrations of FOL4 inoculum in the glasshouse trial, outcomes were not quite as predicted, and the moderate level of inoculum (1×10^5 cfu g^{-1}) did not result in adequate disease levels in the untreated control to evaluate the effect of the biofungicides. This is in contrast to previous experiments carried out at Warwick. While the high level of inoculum (1×10^6 cfu g^{-1}) initiated good disease levels, disease pressure may have been too high for effective control with the products tested. A better approach which is being investigated in AHDB project CP204 may be to use biofungicides to prevent the build-up of FOL4 inoculum in soil while the pathogen is still at modest levels. This could be combined with other approaches such as the use of tolerant or resistant lettuce varieties and possibly soil sterilisation.

Finally, there was no evidence that any of the treatments reduced the level of FOL4 inoculum in the polytunnel soil, as measured by qPCR. Overall, FOL4 DNA levels declined between pre-trial and end-of-trial samples across all treatments and the untreated control. This is difficult to explain as it would be expected that FOL4 levels would increase due to proliferation of the pathogen on lettuce roots during infection. However, although end-of-trial soil samples were collected from near to the lettuce plants, it could be that any additional inoculum from infected roots had not yet been released into the soil.

Polytunnel and Glasshouse Trial Conclusions

- Application of the microbial biofungicides tested did not result in any consistent reduction in lettuce Fusarium wilt in an inoculated trial in the glasshouse.
- Treatments did not reduce the level of FOL4 inoculum in the polytunnel soil, as measured by qPCR.
- Results are in contrast to a trial in 2018 where T34 Biocontrol significantly reduced Fusarium wilt in a similar pot-based trial.
- Other approaches need to be developed for Fusarium wilt control; this could include resistant lettuce cultivars, soil sterilsation or use of microbial biofungicides to prevent build-up of FOL4 inoculum.

Appendix

Trial images

Polytunnel trial

Glasshouse trial

Glasshouse and Polytunnel Trials Diary

Date	Event
23-Jun	Peat blocks prepared with and without treatment
23-Jun	Lettuce seed sown
23-Jun	Plants transferred to GH E8
07-Jul	Phytotoxicity assessment
13-Jul	Trial transplanted into polytunnel
14-Jul	First sprays applied
26-Jul	Second sprays applied
12-Aug	Wilting assessed
18-Aug	Wilting assessed
14-Aug	Wilting assessed
31-Aug	Wilting assessed
12-Sep	Peat blocks prepared with and without treatment
12-Sep	Lettuce seed sown
12-Sep	Plants transferred to GH E8
05-Oct	Phytotoxicity assessment
13-Oct	Trial transplanted into pots
13-Oct	Pots transferred to GH C
13-Oct	First sprays applied
20-Oct	Second sprays applied
27-Oct	Wilting assessed
03-Nov	Wilting assessed
10-Nov	Wilting assessed
17-Nov	Wilting assessed
24-Nov	Wilting assessed
01-Dec	Wilting assessed
02-Dec	Vascular browing assessed

Raw data from assessments

Polytunnel – plot means

Plot	Treatment	12-Aug	18-Aug	24-Aug	31-Aug
1	5	1.43	1.79	2.93	4.07
2	4	2.00	2.21	3.07	4.29
3	2	2.07	2.36	3.71	4.50
4	1	2.07	2.79	3.86	4.71
5	3	2.14	2.50	3.93	4.86
6	2	2.21	2.86	4.07	4.79
7	5	2.14	2.64	4.00	4.71
8	4	2.50	2.79	4.29	5.00
9	1	2.57	2.79	3.86	4.86
10	5	2.36	3.36	4.36	4.86
11	3	2.93	3.21	4.14	4.71
12	2	1.93	2.29	3.57	4.57
13	2	2.50	3.14	4.21	4.86
14	1	3.14	3.93	4.86	5.00
15	4	2.50	3.21	4.21	4.79
16	3	2.71	3.29	4.64	4.86
17	4	2.29	2.64	3.86	4.71
18	3	2.29	2.93	4.14	4.93
19	1	2.71	3.79	4.71	4.93
20	5	2.43	3.29	4.36	4.93

Glasshouse

		Wilting						Browning
Plant	Treatment	27-Oct	03-Nov	10-Nov	17-Nov	24-Nov	01-Dec	02-Dec
1	1	0	0	0	0	0	0	0
2	6	0	0	0	1	2	2	2
3	5	0	0	1	2	2	2	2
4	10	1	2	3	4	5	5	4
5	9	0	2	2	4	4	5	4
6	2	0	0	1	2	2	2	1
7	7	0	2	3	4	4	5	4
8	8	0	2	2	4	4	5	4
9	11	1	2	3	4	4	5	4
10	3	0	0	0	1	2	2	1
11	4	0	1	2	2	3	4	4
12	7	0	2	4	5	5	5	4
13	2	0	0	1	1	2	2	1
14	10	0	2	3	4	5	5	4
15	4	0	1	1	2	2	2	1

16	8	0	1	2	3	4	5	4
17	1	0	0	0	0	0	0	0
18	11	0	1	2	3	4	5	4
19	3	0	2	2	3	3	3	3
20	6	0	0	1	2	2	2	2
21	5	0	1	2	3	4	5	4
22	9	0	1	2	3	3	4	4
23	3	0	0	0	1	2	2	2
24	11	0	1	1	2	2	4	3
25	5	0	0	0	1	2	2	1
26	7	1	2	3	4	5	5	4
27	10	0	1	2	3	4	5	4
28	2	0	1	2	2	2	3	2
29	1	0	0	0	0	0	0	0
30	4	0	0	1	2	2	3	2
31	8	1	2	3	4	5	5	4
32	9	1	2	3	4	4	5	4
33	6	0	0	2	2	2	2	2
34	9	0	0	2	3	4	5	4
35	2	0	0	0	0	1	2	1
36	3	0	0	1	2	2	2	2
37	10	0	2	2	3	4	5	4
38	7	0	1	2	3	4	5	4
39	11	2	3	4	5	5	5	4
40	5	0	1	2	2	2	4	3
41	1	0	0	0	0	0	0	0
42	8	0	2	3	4	4	5	4
43	6	0	3	3	3	4	5	4
44	4	0	2	2	2	2	3	3
45	3	0	0	1	2	3	4	3
46	6	0	0	1	2	3	3	3
47	10	1	1	2	3	4	5	4
48	2	0	0	1	1	2	2	2
49	7	0	1	2	3	4	5	4
50	9	0	2	2	3	4	5	4
51	5	0	0	1	2	3	4	3
52	4	0	1	1	2	2	2	2
53	8	0	2	3	4	4	5	4
54	11	0	2	2	3	3	4	3
55	1	0	0	0	0	0	0	0
56	1	0	0	0	0	0	0	0
57	9	0	2	3	4	5	5	4
58	10	1	2	4	4	5	5	4
59	5	0	1	2	3	5	5	4

60	2	0	1	1	2	2	2	1
61	3	0	1	2	2	3	4	3
62	4	0	0	1	2	2	2	2
63	11	0	1	2	3	4	5	4
64	6	0	0	1	2	2	3	3
65	8	0	1	2	3	4	5	4
66	7	1	1	2	4	5	5	4
67	10	1	2	3	4	4	5	4
68	3	0	1	2	2	2	3	3
69	5	0	1	2	2	2	2	2
70	1	0	0	0	0	0	0	0
71	8	0	2	2	4	5	5	4
72	7	0	1	2	3	4	5	4
73	6	0	0	2	2	2	2	2
74	9	0	0	2	4	4	5	4
75	4	0	0	0	1	2	2	1
76	11	0	0	1	2	2	3	2
77	2	0	1	1	1	2	2	1
78	8	0	1	2	2	3	4	4
79	3	0	2	3	3	3	5	4
80	1	0	0	0	0	0	0	0
81	10	0	1	2	3	4	5	4
82	7	1	2	3	4	5	5	4
83	4	0	0	1	2	2	2	1
84	9	1	2	4	4	5	5	4
85	2	0	0	1	2	2	2	1
86	6	0	1	2	2	3	4	3
87	11	0	2	3	4	5	5	4
88	5	0	1	2	2	2	2	3
89	10	0	2	3	4	5	5	4
90	9	0	2	3	4	5	5	4
91	7	1	2	3	4	4	5	4
92	5	0	1	2	2	3	3	2
93	4	0	0	1	2	2	2	1
94	11	0	1	2	3	4	5	4
95	3	0	1	1	2	3	3	3
96	1	0	0	0	0	0	0	0
97	6	0	0	1	2	3	4	3
98	8	0	2	2	3	4	4	4
99	2	0	0	1	2	2	2	1
100	2	0	0	1	2	2	2	2
101	10	0	2	3	3	3	4	3
102	1	0	0	0	0	0	0	0
103	9	1	2	2	3	4	5	4

104	5	0	1	2	3	4	5	4
105	7	0	2	2	4	5	5	4
106	3	0	1	1	2	2	2	1
107	8	0	1	1	2	3	4	4
108	6	0	1	1	2	2	2	1
109	4	0	0	1	2	2	2	2
110	11	0	2	4	4	5	5	4
111	11	0	1	3	4	5	5	4
112	5	0	0	1	2	2	2	2
113	4	0	1	2	3	3	3	3
114	8	0	1	2	3	4	5	4
115	9	0	2	2	3	4	5	4
116	6	0	0	1	1	2	2	1
117	7	0	1	2	4	5	5	4
118	1	0	0	0	0	0	0	0
119	10	0	1	2	2	4	5	4
120	2	0	1	1	2	2	2	1
121	3	0	0	0	2	2	2	1
122	2	0	0	0	0	1	2	1
123	1	0	0	0	0	1	1	0
124	3	0	0	0	2	2	2	2
125	10	1	2	2	4	4	5	4
126	11	0	0	1	2	2	4	3
127	8	0	2	3	4	4	5	4
128	4	0	1	2	2	2	2	2
129	5	0	0	0	1	2	2	1
130	6	0	0	1	2	2	2	1
131	9	0	1	2	3	3	5	4
132	7	1	2	3	4	5	5	4
133	10	0	1	1	2	4	5	4
134	4	0	0	0	1	2	4	3
135	5	0	0	0	1	1	2	1
136	1	0	0	0	0	0	0	0
137	2	0	0	0	1	2	3	2
138	9	1	2	3	4	4	5	4
139	11	0	2	2	4	4	5	4
140	3	0	0	0	0	2	2	1
141	8	0	1	2	4	5	5	4
142	6	0	1	1	2	2	3	2
143	7	0	2	3	4	4	5	4
144	11	0	0	1	3	4	5	4
145	7	0	2	2	2	2	3	2
146	9	0	0	2	3	4	5	4
147	10	0	0	1	2	3	5	4

148	4	0	2	2	2	2	3	2
149	2	0	0	0	1	2	2	1
150	3	0	0	0	1	2	2	1
151	1	0	0	0	0	0	0	0
152	8	0	1	2	2	3	4	4
153	5	0	0	1	2	2	3	2
154	6	0	0	1	2	2	2	3
155	2	0	0	1	1	1	2	1
156	5	0	1	3	4	4	5	4
157	1	0	0	0	0	0	0	0
158	3	0	0	0	1	2	2	2
159	11	0	0	2	3	3	4	4
160	7	1	3	4	4	5	5	4
161	9	1	2	3	4	5	5	4
162	8	0	2	2	4	5	5	4
163	6	0	2	3	4	5	5	4
164	4	0	0	1	1	2	2	2
165	10	0	2	2	2	3	4	3
166	11	0	1	2	3	5	5	4
167	1	0	0	0	0	0	0	0
168	10	0	2	3	4	5	5	4
169	7	0	2	2	4	5	5	4
170	9	0	2	4	4	5	5	4
171	8	0	1	2	3	4	5	4
172	2	0	2	2	3	4	5	4
173	3	0	1	1	2	2	2	3
174	6	0	1	1	2	2	2	2
175	4	0	1	1	2	2	2	1
176	5	0	1	2	2	2	3	2
177	6	0	0	1	2	2	3	3
178	8	1	2	3	4	5	5	4
179	4	0	1	1	2	2	3	3
180	1	0	0	0	0	0	0	0
181	7	0	1	2	3	4	5	4
182	5	0	0	2	2	2	3	2
183	10	0	2	2	2	3	4	3
184	3	0	0	1	2	2	2	1
185	9	0	2	2	3	4	5	4
186	11	0	2	2	3	4	5	4
187	2	0	1	2	2	2	3	3
188	1	0	0	0	0	0	0	0
189	5	0	1	2	2	2	3	2
190	11	0	0	2	2	3	4	3
191	8	0	1	2	4	5	5	4

192	6	0	0	1	2	2	2	1
193	2	0	0	0	1	2	2	2
194	4	0	1	2	2	2	2	3
195	3	0	0	1	1	2	2	1
196	9	0	2	3	4	5	5	4
197	7	0	1	2	3	4	4	4
198	10	0	1	2	3	3	4	4
199	7	0	2	2	3	4	5	4
200	11	1	2	2	3	4	5	4
201	6	0	2	2	2	3	4	4
202	3	0	2	2	2	4	5	4
203	9	1	2	3	4	5	5	4
204	1	0	0	0	0	0	0	0
205	2	0	0	2	2	2	2	1
206	10	0	2	2	4	5	5	4
207	8	0	2	2	4	5	5	4
208	4	0	0	0	2	2	2	1
209	5	0	0	1	2	2	3	3
210	3	0	0	0	1	2	2	1
211	2	0	0	1	1	2	2	1
212	10	0	1	2	3	4	5	4
213	6	0	1	2	2	3	4	3
214	11	1	2	2	4	4	5	4
215	7	0	1	2	3	4	5	4
216	8	1	2	2	3	4	5	4
217	5	0	0	2	2	2	2	2
218	1	0	0	0	0	0	0	0
219	9	0	2	3	4	5	5	4
220	4	0	0	0	1	2	3	2

Design

Polytunnel

1	4	2	3	5
2	5	3	4	1

Door

4	2	5	1	3
5	3	1	2	4

1 Untreated control
2 T34
3 Trianum P
4 Prestop
5 Serifel

Glasshouse

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

Block	6	7	8	9	10	16	17	18	19	20
	1	10	8	10	2	11	6	1	7	3
	9	3	3	9	10	1	8	5	11	2
	10	5	1	7	1	10	4	11	6	10
	5	1	10	5	9	7	1	8	3	6
	2	8	7	4	5	9	7	6	9	11
	3	7	4	11	7	8	5	2	1	7
	4	6	9	3	3	2	10	4	2	8
	11	9	2	1	8	3	3	3	10	5
	6	4	6	6	6	6	9	9	8	1
	8	11	11	8	4	4	11	7	4	9
	7	2	5	2	11	5	2	10	5	4

Treat. No.	Product	FOL4 inoculum level
1	Untreated	None
2	Untreated	Moderate
3	T34	Moderate
4	Prestop	Moderate
5	Trianum	Moderate
6	Serifel	Moderate
7	Untreated	High
8	T34	High
9	Prestop	High
10	Trianum	High
11	Serifel	High

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