SCEPTREPLUS

Final Trial Report

Trial code:	SP 40
Title:	Control of new tomato blight strain
Crop	Group; Protected edibles - tomato
Target	*Phytopthora infestans* EU39_A1 strain
Lead researcher:	Dave Kaye
Organisation:	RSK ADAS Ltd.
Period:	June 2019 – September 2019
Report date:	30 November 2019
Report author:	Dave Kaye
ORETO Number: (certificate should be attached)	ORETO 409

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

Date: 30 November 2019 Authors signature: D. Kaye.

Trial Summary

Introduction

Phytophthora infestans, an oomycete, is the causal agent of late blight in tomato. *P. infestans* typically manifests towards the end of the tomato growing season, affecting the aerial parts of plants. In January 2018 a new strain of *P. infestans* (EU39_A1 genotype) was identified by the James Hutton Institute (JHI), occurring on multiple UK production sites. This strain was demonstrated to colonise the roots, as well as aerial parts of the plants, developing from the graft wound, likely from the presence of the pathogen at the grafting stage. The infection was present on multiple production sites which all sourced their plants from the same propagator. This outbreak resulted in the death of several thousand young plants, however no new infections developed, with only plants affected before arrival on site being affected. Surviving plants continued to exhibit symptoms, as stem lesions, despite chemical treatment with Ranman Top (cyazofamid). Lesions spread along the stems, typically from the site of the grafting wound. Few products are currently registered against late blight in tomato and these have a limited number of applications authorised. New products are therefore needed to provide UK growers with effective treatments against *P. infestans* for the entire growing season.

Methods

Due to biosecurity, costs and the high risk of placing a *P. infestans* trial on a commercial site, a polytunnel trial based at ADAS Boxworth (Cambridgeshire) was established. A four block, randomised fungicide efficacy trial, using a known *P. infestans* susceptible tomato variety (Elegance) was set up, and artificially inoculated using an isolate of *P. infestans* EU39_A1. Young plants were grown at a local propagator in rockwool cubes, before being planted onto rockwool slabs, and strung for support. The crop was fertigated utilising a Dosatron and drip peg irrigation system and treated according to commercial standards, de-leafing as necessary, with fruit harvested on two occasions. Biocontrols were introduced to manage pests, and no outbreaks developed. No fungicide inputs were required in addition to the test products.

Each treatment plot within each of the four blocks comprised of four tomato plants (single head), split across two slabs to give a total of 16 plants per treatment. Twenty four hours prior to inoculation, the fourth true leaf from each plant was cut away using a new, sterilised scalpel blade, which was re-sterilised between each cut using 70% industrial methylated spirit (IMS), leaving a wound at the petiole base close to the stem. The stem lesions seen in commercial plants have developed from the site of grafting, and so the wounding carried out in this experiment replicated this infection pathway. Both sides of the plant, including the wound site, were then initially treated preventatively with the test products, apart from AHDB9941, which arrived on the day of inoculation and was therefore applied one day after. Subsequently, all products were applied a further three times as curative treatments.

On 1 August, the day after initial treatment application, a 4 mm plug of *P infestans* (EU39_A1 strain), from a two week pea:rye agar culture, containing mature sporangia, was placed hyphal side down onto the pre-moistened stem wound on each plant. The agar plug and stem surrounding the wound were wrapped in plastic film to ensure they remained in place and relative humidity levels remained high. The film wrapping the plugs was then removed 24 hours later. Following artificial inoculation, the doors and sides of the polytunnel remained closed for biosecurity concerns, with limited access to staff. The floor of the polytunnel was wetted down (three times per day) for the first seven days, to maintain high relative humidity and maximise disease pressure.

Products were applied a total of four times to each plot using a knapsack sprayer, at roughly 10 day intervals (31/07/19, 09/08/19, 19/08/19 and 29/08/19). Plants were assessed for disease seven times from the first treatment application to 14 days following the final application. At each assessment, the incidence and severity of *P. infestans* symptoms were assessed. Incidence was recorded as presence or absence of *P. infestans* per plant (1 or 0, respectively). Severity was scored as the maximum length (mm) of the stem lesion which

developed from the original wound site using calibrated digital calipers. In addition to this, crop safety was recorded with any symptoms attributed to phytotoxicity noted (0-10 scale where 0 = dead, 10 = no damage).

Results

Sufficient, and consistent levels of disease due to *P. infestans* inoculation developed which allowed differences to be observed between treated and the untreated plants. All plants in the untreated inoculated control plots developed symptoms while there was variation in disease levels within fungicide-treated inoculated plants. *P. infestans* stem lesions developed by the first full disease assessment (five days after inoculation) and continued to expand, with an average lesion length in the untreated of 95.4 mm by the final assessment. The industry standard Ranman Top provided no significant control of *P. infestans* at any assessment date corresponding with the reduced sensitivities reported by growers.

Disease Incidence: No test product completely prevented disease establishment and symptoms development in almost all treated plants. Only one product, AHDB9841, resulted in significant reductions in disease incidence compared with the untreated control, from five days after inoculation (d.a.i) onwards (Table 1).

Table 1. Effect of different fungicide treatments on mean *P. infestans* disease incidence (% of plants per plot affected) for each of seven assessment dates.

Date / Treatment	31-July	05-Aug	09-Aug	14-Aug	19-Aug	03-Sep	17-Sep
Untreated	0.0	100.0	100.0	100.0	100.0	100.0	100.0
Ranman Top	0.0	100.0	100.0	100.0	100.0	100.0	100.0
Carial Star	0.0	93.8	93.8	93.8	93.8	93.8	93.8
AHDB9879	0.0	100.0	100.0	100.0	100.0	100.0	100.0
AHDB9882	0.0	87.5	87.5	87.5	87.5	87.5	87.5
AHDB9852	0.0	100.0	100.0	100.0	100.0	100.0	100.0
AHDB9959	0.0	100.0	100.0	100.0	100.0	100.0	100.0
AHDB9967	0.0	87.5	87.5	87.5	87.5	87.5	87.5
AHDB9941	0.0	93.8	93.8	93.8	93.8	93.8	93.8
AHDB9841	0.0	62.5	62.5	62.5	62.5	62.5	62.5
	Not significantly different from untreated control (p>0.05)						
	Significantly different from untreated control (p<0.05)						

Disease Severity: Seven of the nine test products (Carial Star, AHDB9841, AHDB9882, AHDB9941, AHDB9879, AHDB9959 and AHDB9967) significantly reduced disease severity on at least one assessment date compared with the untreated control. Only AHDB9841 resulted in reductions in severity from assessment 2, until the end of the trial (Table 2).

Table 2. Effect of different fungicide treatments on mean *P. infestans* disease severity (lesion length, mm) for each of seven assessment dates.

Date / Treatment	31-July	05-Aug	09-Aug	14-Aug	19-Aug	03-Sep	17-Sep
Untreated	0.0	18.5	43.4	61.8	83.7	90.9	95.4
Ranman Top	0.0	12.6	27.9	47.1	69.1	78.9	86.1
Carial Star	0.0	8.2	14.8	24.7	41.5	50.1	55.3
AHDB9879	0.0	10.4	12.9	22.8	36.9	47.3	54.2
AHDB9882	0.0	7.0	13.8	23.0	39.1	48.3	51.6
AHDB9852	0.0	16.1	42.7	77.9	108.1	130.0	139.1
AHDB9959	0.0	10.8	30.0	51.4	73.5	86.2	93.9
AHDB9967	0.0	11.8	19.4	23.5	41.8	50.2	58.8
AHDB9941	0.0	8.9	20.1	31.7	38.6	45.7	60.3
AHDB9841	0.0	5.8	6.7	8.3	9.8	10.1	11.6
	Not significantly different from untreated control (p>0.05)						
	Significantly different from untreated control (p<0.05)						

Phytotoxicity
No visible symptoms of phytotoxicity developed on any of the tomato plants after treatment, with any product, at any assessment date. However, minor symptoms of nutritional deficiency developed as a consequence of an issue with the Dosatron used for managing fertigation. No differences in deficiency symptoms were apparent between treated and untreated plants at any time.

Conclusions

- The industry standard, Ranman Top, did not reduce the incidence or severity of disease due to *P. infestans*, compared with the untreated control.
- AHDB9841 alone, applied preventatively, and then curatively three times during cropping, significantly reduced incidence of *P. infestans* compared with the untreated control.
- Seven of the nine test products (Carial Star, AHDB9841, AHDB9882, AHDB9941, AHDB9879, AHDB9959 and AHDB9967) significantly reduced disease severity on at least one assessment date, but had no effect on the proportion of plants affected.
- AHDB9879 and AHDB9882 performed similarly well to Carial Star, but have different modes of action, and could be a valuable tool in resistance management.
- The biological product AHDB9967 significantly reduced disease severity at two assessments and could be used in organic production.
- AHDB9941 was only applied curatively, but is known to have protectant properties.
- AHDB9841 alone resulted in reductions in disease severity from 5 d.a.i. until the end of the trial compared with the untreated control.
- AHDB9841 resulted in reductions in disease severity compared with the industry standard, Ranman Top, from 5 d.a.i until the end of the trial after six weeks.
- No symptoms associated with phytotoxicity developed on any plants, at any date.

Take home message:

Although the four applications of seven of the nine test products reduced *P. infestans* lesion size on at least one assessment date, only AHDB9841 successfully, and consistently, reduced both disease incidence and severity compared with the untreated control.

Objective

To assess a range of fungicides, for their safety and efficacy against the new strain of *Phytopthora infestans* (EU39_A1).

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 1/135(4)	Phytotoxicity assessment	None
PP 1/152(4)	Guideline on design and analysis of efficacy evaluation trials	None
PP 1/225(2)	Minimum effective dose	None
PP 1/181(4)	Conduct and reporting of efficacy evaluation trials including good experimental practice	None
PP 1/214(3)	Principles of acceptable efficacy	None
PP 1/224(2)	Principles of efficacy evaluation for minor uses	None

A plot size of four plants/plot was used due to size constraints within the pathogen containment area of the polytunnel. Each plant was artificially inoculated guaranteeing infection.

Test site

Item	Details
Location address	Polytunnel 5 ADAS Boxworth Battlegate Road Boxworth Cambs CB23 4NN Grid reference: TL 34359 63381
Crop	Tomato
Cultivar	Elegance
Soil or substrate type	Grodan Vital rockwool slabs
Agronomic practice	Normal, strung, fertigated crop, regularly de-leafed and harvested twice, no CO_2 enrichment.
Prior history of site	N/A

Trial design

Item	Details
Trial design:	Randomised block
Number of replicates:	4
Row spacing:	1.5 m
Plot size: (w x l)	2.4 m x 0.15 m
Plot size: (m^2)	0.36 m^2
Number of plants per plot:	4
Leaf Wall Area calculations	N/A

Treatment details

AHDB Code	Active substance	Product name/ manufacturers code	Formulation batch number	Content of active substance in product	Formulation type
N/A	Water	Untreated	N/A	N/A	N/A
Approved	Cyazofamid	Ranman Top	18008885/4	160 g L^{-1}	Suspension concentrate
Approved	Difenoconazole/ mandipropamid	Carial Star	GRA7D211E	250:250 g L^{-1}	Suspension concentrate
AHDB9879	N/D	N/D	N/D	N/D	N/D
AHDB9882	N/D	N/D	N/D	N/D	N/D
AHDB9852	N/D	N/D	N/D	N/D	N/D
AHDB9959	N/D	N/D	N/D	N/D	N/D
AHDB9967	N/D	N/D	N/D	N/D	N/D
AHDB9941	N/D	N/D	N/D	N/D	N/D
AHDB9841	N/D	N/D	N/D	N/D	N/D

No adjuvants were included at any treatment application.
*N/D = content of active ingredient/s not disclosed by manufacturer

Application schedule

Treatment number	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	Control (water)	-	-	A-D
2	Ranman Top	80.00	0.50	A-D
3	Carial Star	150.00 & 150.00	0.60	A-D
4	AHDB9879	N/D	0.70	A-D
5	AHDB9882	N/D	1.60	A-D
6	AHDB9852	N/D	3.20	A-D
7	AHDB9959	N/D	2.50	A-D
8	AHDB9967	N/D	3.20	A-D
9	AHDB9941	N/D	2.50	A-D
10	AHDB9841	N/D	0.50	A-D

Application details

	Application A	Application B	Application C	Application D
Application date	31/07/2019	09/08/2019	19/08/2019	29/08/2019
Time of day	14:00 - 14:35	16:30 - 17:00	16:00 - 16:30	15:00 - 15:40
Crop growth stage (Max, min average BBCH)	702	702	703	703
Crop height (cm)	90-100 cm	110-120 cm	120-140 cm	150-170 cm
Crop coverage (%)	80	80	80	80
Application Method	Spray	Spray	Spray	Spray
Application Placement	Foliar	Foliar	Foliar	Foliar
Application equipment	Oxford Precision Sprayer (Knapsack)	Oxford Precision Sprayer (Knapsack)	Oxford Precision Sprayer (Knapsack)	Oxford Precision Sprayer (Knapsack)
Nozzle pressure (bar)	2.0	2.0	2.0	2.0
Nozzle type	Flat fan	Flat fan	Flat fan	Flat fan

	Application A	Application B	Application C	Application D
Nozzle size	02F110	02F110	02F110	02F110
Application water volume/ha	400	400	400	400
Temperature of air - shade (°C)	26.5 - 22.5	37.0 - 38.5	34.0 – 31.0	35.5 - 26.5
Relative humidity (%)	55.0 – 63.0	54.5 - 46.5	43.0 - 41.5	40.0-61.0
*Wind speed range (m/s)	N/A	N/A	N/A	N/A
Dew presence (Y/N)	N	N	N	N
*Temperature of soil - 2-5 cm (°C)	N/A	N/A	N/A	N/A
*Wetness of soil - 2-5 cm	N/A	N/A	N/A	N/A
*Cloud cover (%)	N/A	N/A	N/A	N/A

*The crop was grown under protection in artificial substrate. Soil temperature, wetness, cloud cover and wind speed were not recorded.
**AHDB9941 arrived a day late and was applied on 02/08/19. The three remaining treatment applications were applied at the same dates as applications B-D.

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

Common name	Scientific Name	EPPO Code	Infestation level pre-application	Infestation level at start of assessment period	Infestation level at end of assessment period
Late Blight	*Phytopthora infestans* (EU39_A1 strain)	PHYTIN	0%	100% incidence Lesion length – 18.5 mm	100% incidence Lesion length – 95.4 mm

No pests/pest damage were recorded on any plants at any assessment date

Artificial inoculation

P. infestans strain EU39_A1 taken from the ADAS refrigerated long-term culture collection was subbed onto 20 plates of pea:rye agar (15/07/2019). Plates were incubated at 20°C for 17 days, allowing time for colony growth. The presence of mature sporangia, capable of releasing infectious zoospores, was confirmed via microscopy and the trial artificially inoculated on 01/08/2019.

Plants were wounded by the removal of the fourth true leaf using a scalpel sterilised in 70% IMS solutions 24 hours before inoculation leaving a 0.5cm leaf stub which received treatment with the fungicides tested shortly after. Four plates were selected, based on their similarities in fungal colony size and morphology

The pre-treated wound sites were moistened with three pumps of sterile distilled water from a hand held pump immediately before individual 4 mm diameter plugs, taken from the agar plates, were placed ‘fungal mycelium side down’ onto them. These plugs were lightly pressed onto to the wound site to ensure adhesion, and the stems wrapped in plastic film for 24 h to maintain high relative humidity and aid infection. The test product AHDB9941 did not arrive until the day after inoculation, and hence was applied curatively 24 hours after artificial inoculation.

Assessment details

A preliminary disease assessment was performed on 31/07/2019, immediately before the first treatment application. Six additional assessments were subsequently completed, corresponding with the later treatment dates and 14 days after the final treatment application. Two disease assessments were also carried out between the first and second treatment application dates to more closely gauge the effectiveness of each of the treatments.

At every assessment, each tomato plant was assessed for *P. infestans* incidence as the proportion of plants with stem lesions present while severity was recorded as the length of

developing stem legions measured using digital calipers (mm). In addition to these assessments, a lateral flow diagnostic device (LFD) specific to *Phytopthora* spp. was used to confirm the presence of the pathogen.

Crop safety effects were noted and symptoms recorded on a whole plot scale These were scored on a scale from 0 to 10, with 0 being 'dead', and 10 being 'no effect'. Plots which scored 8 or above were deemed to have a commercially acceptable level of damage.

Phytotoxicity was recorded using the following scale:

Table 3. Scale used for the assessment of the extent of phytotoxic damage in treated plots

Crop tolerance score	Equivalent to crop damage (% phytotoxicity)
0	complete crop kill 100%
1	80-95% damage
2	70-80%
3	60-70%
4	50-60%
5	40-50%
6	25-40%
7	15-25%
8*	10-15%
9	5-10%
10	no damage

* 8 = acceptable damage, i.e. damage unlikely to reduce yield, and acceptable to the grower.

Table 4. *P. infestans* disease and crop safety assessment schedule

Evaluation date	Evaluation Timing (DA)*	Crop Growth Stage (BBCH)	Evaluation type (efficacy, phytotoxicity)	Assessment
31/07/2019	1	702	Preliminary	- *P. infestans* incidence and severity
01/08/2019	**2**	**702**	**-**	**- Inoculation date**
05/08/2019	5	702	Efficacy Phytotoxicity	- *P. infestans* incidence and severity - Crop safety
09/08/2019	10	702	Efficacy Phytotoxicity	- *P. infestans* incidence and severity - Crop safety
14/08/2019	14	703	Efficacy Phytotoxicity	- *P. infestans* incidence and severity - Crop safety
19/08/2019	20	703	Efficacy Phytotoxicity	- *P. infestans* incidence and severity - Crop safety
29/08/2019	30	703	Efficacy Phytotoxicity	- *P. infestans* incidence and severity - Crop safety
13/09/2019	45	704	Efficacy Phytotoxicity	- *P. infestans* incidence and severity - Crop safety

* DA – days after first spray application.

Statistical analysis

The trial was analysed by Chris Dyer (ADAS statistician) as a randomised block design with four replicates of 10 treatments using ANOVA (Genstat 18th edition). No data transformation was required.

Results

Phytotoxicity

No test treatments resulted in any phytotoxic effects on stems, leaves, fruit or flowers with all plots scoring 10 (no damage).

Table 5. Phytotoxicity scores for six assessment dates of plots treated with test products.

Date / Treatment	05-Aug	09-Aug	14-Aug	19-Aug	29-Aug	13-Sep
Untreated	10.00	10.00	10.00	10.00	10.00	10.00
Ranman Top	10.00	10.00	10.00	10.00	10.00	10.00
Carial Star	10.00	10.00	10.00	10.00	10.00	10.00
AHDB9879	10.00	10.00	10.00	10.00	10.00	10.00
AHDB9882	10.00	10.00	10.00	10.00	10.00	10.00
AHDB9852	10.00	10.00	10.00	10.00	10.00	10.00
AHDB9959	10.00	10.00	10.00	10.00	10.00	10.00
AHDB9967	10.00	10.00	10.00	10.00	10.00	10.00
AHDB9941	10.00	10.00	10.00	10.00	10.00	10.00
AHDB9841	10.00	10.00	10.00	10.00	10.00	10.00

* 8 = acceptable damage, i.e. damage unlikely to reduce yield, and considered commercially acceptable.

Efficacy

Disease incidence: Artificial inoculation with the *P. infestans* was confirmed using a generic Phytopthora LFD and disease symptoms developed in all treatments. No sporulation occurred on any of the inoculated plants and as such, no secondary lesions developed. Differences in the incidence of *P. infestans* were observed between AHDB9841 and the untreated control within five days of artificial inoculation, (Table 6). AHDB9841 also significantly reduced disease incidence compared to the industry standard Ranman Top. No other treatment significantly reduced disease incidence at any assessment date (Table 6).

Table 6. Effect of different fungicide treatments on mean *P. infestans* disease incidence (% of plants per plot affected) for each of seven assessment dates.

Date / Treatment	31-July	05-Aug	09-Aug	14-Aug	19-Aug	03-Sep	17-Sep
Untreated	0.0	100.0	100.0	100.0	100.0	100.0	100.0
Ranman Top	0.0	100.0	100.0	100.0	100.0	100.0	100.0
Carial Star	0.0	93.8	93.8	93.8	93.8	93.8	93.8
AHDB9879	0.0	100.0	100.0	100.0	100.0	100.0	100.0
AHDB9882	0.0	87.5	87.5	87.5	87.5	87.5	87.5
AHDB9852	0.0	100.0	100.0	100.0	100.0	100.0	100.0
AHDB9959	0.0	100.0	100.0	100.0	100.0	100.0	100.0
AHDB9967	0.0	87.5	87.5	87.5	87.5	87.5	87.5

AHDB9941	0.0	93.8	93.8	93.8	93.8	93.8	93.8
AHDB9841	0.0	62.5	62.5	62.5	62.5	62.5	62.5
P value	-	0.013	0.013	0.013	0.013	0.013	0.013
d.f.	-	27	27	27	27	27	27
s.e.d.	-	9.56	9.56	9.56	9.56	9.56	9.56
l.s.d.	-	19.62	19.62	19.62	19.62	19.62	19.62
	Not significantly different from untreated control (p>0.05)						
	Significantly different from untreated control (p<0.05)						

Disease severity: Statistical differences in disease severity between the untreated control and fungicide treated plants was evident at assessment 2 (05/08/2019, Table 7) and at the following assessment on 09/08/2019, six products, Carial Star, AHDB9879, AHDB9882, AHDB9967, AHDB9941 and AHDB9841 significantly reduced disease severity. AHDB9959 only reduced disease severity at assessment 2. AHDB9967 had no impact at assessment 2, but reduced severity at assessment 3 (09/08/2019). Carial Star, AHDB9879, AHDB9882, AHDB9967 and AHDB9841 also all reduced disease severity at assessment 4 (14/08/2019). After this date, only AHDB9841 reduced disease severity compared to the untreated control (11.6 mm lesion length in AHDB9841 treated plants vs. 95.4 mm in untreated plants, at the final assessment date). Plants treated with AHDB9841 also had significantly reduced disease severity compared with the industry standard, Ranman Top, from assessment 3 onwards.

The use of AHDB9852 appeared to increase the size of the stem lesions of the inoculated plants (139.1 mm in AHDB9852 treated plants vs. 95.4 mm in untreated plants, at the final assessment date) although this was not statistically significant.

Table 7. Effect of different fungicide treatments on mean *P. infestans* disease severity (lesion length, mm) for each of seven assessment dates.

Date Treatment	31-July	05-Aug	09-Aug	14-Aug	19-Aug	03-Sep	17-Sep
Untreated	0.0	18.5	43.4	61.8	83.7	90.9	95.4
Ranman Top	0.0	12.6	27.9	47.1	69.1	78.9	86.1
Carial Star	0.0	8.2	14.8	24.7	41.5	50.1	55.3
AHDB9879	0.0	10.4	12.9	22.8	36.9	47.3	54.2
AHDB9882	0.0	7.0	13.8	23.0	39.1	48.3	51.6
AHDB9852	0.0	16.1	42.7	77.9	108.1	130.0	139.1
AHDB9959	0.0	10.8	30.0	51.4	73.5	86.2	93.9
AHDB9967	0.0	11.8	19.4	23.5	41.8	50.2	58.8
AHDB9941	0.0	8.9	20.1	31.7	38.6	45.7	60.3
AHDB9841	0.0	5.8	6.7	8.3	9.8	10.1	11.6
P value	-	0.007	<0.001	0.001	0.003	0.004	0.007
d.f.	-	27	27	27	27	27	27
s.e.d.	-	3.08	7.55	14.46	20.67	24.54	26.51
l.s.d.	-	6.31	15.49	29.68	42.41	50.35	54.40
	Not significantly different from untreated control (p>0.05)						
	Significantly different from untreated control (p<0.05)						

Figure 1 shows the results from disease severity assessments 2-5, with orange bars representing significant reductions in stem lesion length compared with the untreated control. It is clear that despite several products resulting in reduction in stem lesion length at different assessment times, only AHDB9841 had a consistent effect. Carial star, AHDB9879, AHDB9882 and to some extent AHDB9967 and AHDB9941 all performed comparably well at the first three assessments.

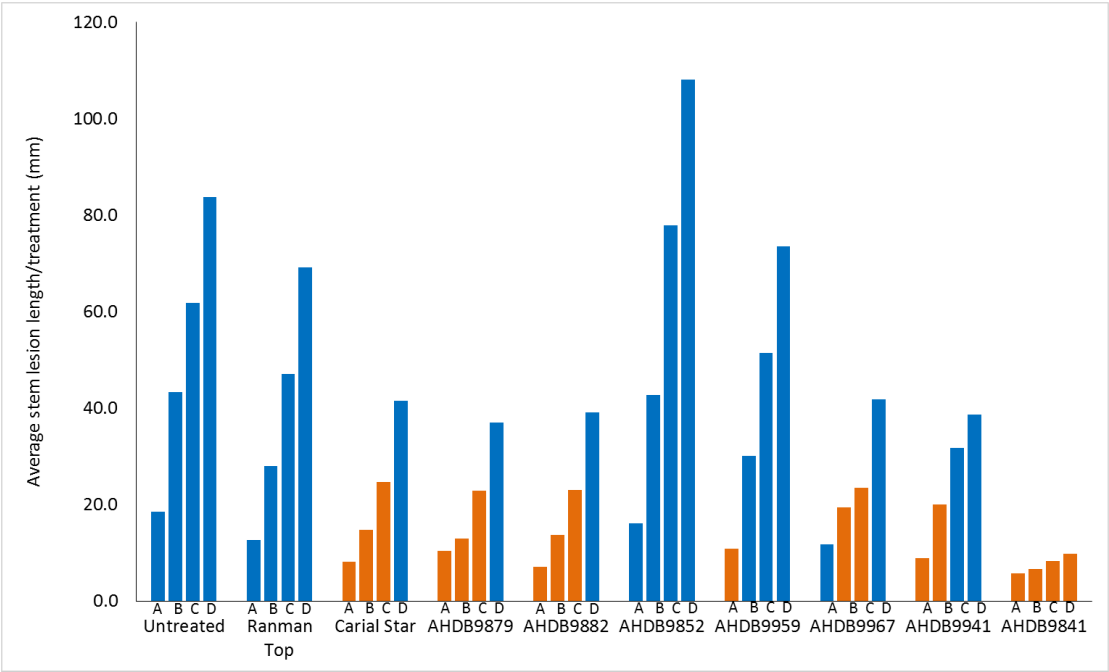

Figure 1. Effect of fungicide treatments on mean stem lesion length (mm) in a tomato crop artificially inoculated with *P. infestans* at four assessment dates, A (05-Aug), B (09-Aug), C (14-Aug) and D (19-Aug).
*Orange bars represent significant reductions, ($p<0.05$), in stem lesion length compared with the untreated control.

Figure 2 shows the results of stem lesion length at the final assessment on 17/09/19, after all plots had received four treatment applications. This clearly shows that only AHDB9841 significantly reduced symptoms at this time after the final applications on 29/08/2019.

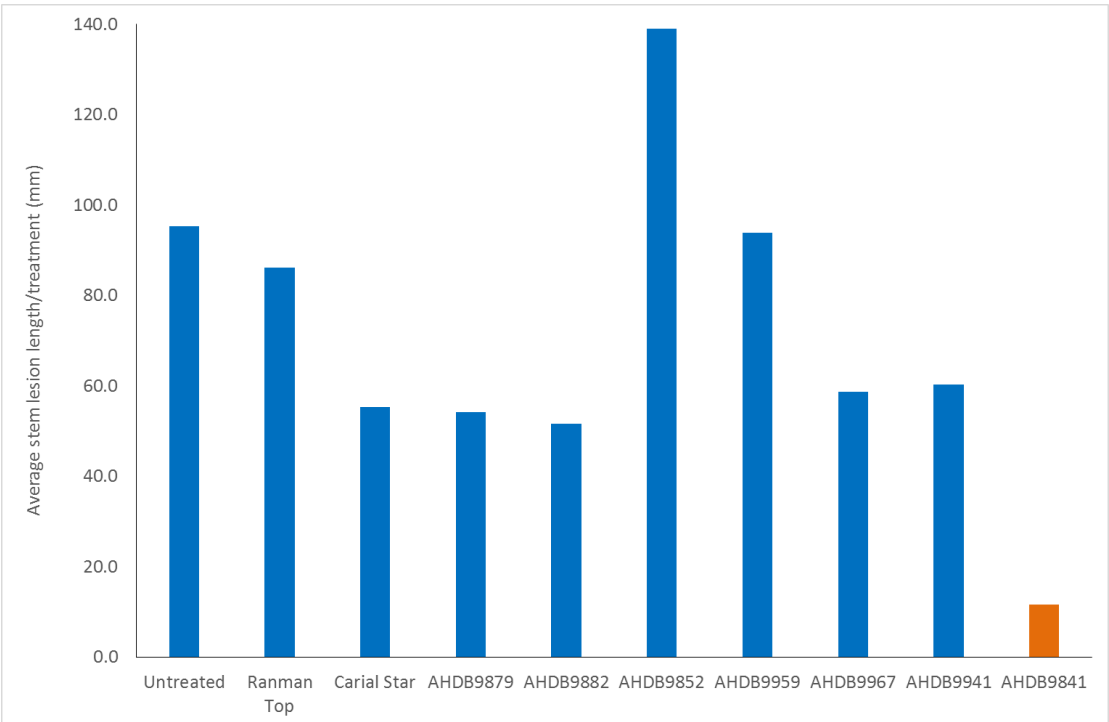

Figure 2. Effect of fungicide treatments on mean stem lesion length (mm) in a tomato crop artificially inoculated with *P. infestans* at the final assessment date (17/09/2019).
*Orange bar represents significant reductions ($p<0.01$) in stem lesion length compared with the untreated control.

Percentage reduction in stem lesion length for each treatment compared with the untreated control at each assessment date is shown in Table 8. This clearly indicates the products resulting in the greatest reductions in lesion growth and the consistent performance of AHDB9841.

Table 8. Percentage reduction in *P. infestans* stem lesion length compared to to the untreated control for different fungicide treatments and assessment dates.

Date / Treatment	31-July	05-Aug	09-Aug	14-Aug	19-Aug	03-Sep	17-Sep
Untreated	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ranman Top	0.0	31.9	35.7	23.8	17.4	13.2	9.7
Carial Star	0.0	**55.7**	**65.9**	**60.0**	50.4	44.9	42.0
AHDB9879	0.0	**43.8**	**70.3**	**63.1**	55.9	48.0	43.2
AHDB9882	0.0	**62.2**	**68.2**	**62.8**	53.3	46.9	45.9
AHDB9852	0.0	13.0	1.6	-26.1	-29.2	-43.0	-45.8
AHDB9959	0.0	**41.6**	30.9	16.8	12.2	5.2	1.6
AHDB9967	0.0	36.2	**55.3**	**62.0**	50.1	44.8	38.4
AHDB9941	0.0	**51.9**	**53.7**	48.7	53.9	49.7	36.8
AHDB9841	0.0	**68.6**	**84.6**	**86.6**	**88.3**	**88.9**	**87.8**

Values in bold correspond to results significantly different compared with the untreated control ($p<0.05$).

Discussion

Consistent levels of disease due to *P. infestans* developed in the trial allowing differences between treatments and the untreated control to be observed. All plants in the untreated control plots developed typical stem lesion symptoms. No sporulation was observed on any stem lesions meaning that there was no inoculum source for further spread; hence no secondary lesions developed on any of the plants. This was consistent with what was seen on commercial production sites in 2018. Following inoculation, *P. infestans* stem lesions developed rapidly, appearing by the first full disease assessment four days later. Lesions continued to expand, with the average lesion length in the untreated control reaching 95.4 mm by the final assessment. There were no significant differences in the length of stem lesions between the untreated and industry standard, Ranman Top, at any assessment date. These results match with reports of a reduced sensitivity of the EU39_A1 strain to Ranman Top, and this might explain the results seen. Decreased sensitivity to Ranman Top would need to be investigated *in vitro* with other isolates of *P. infestans*.

AHDB9841 (FRAC code 40) was the only product to reduce *P. infestans* incidence compared with the untreated control. This is unsurprising as this product is already authorised for use against *P. infestans* in potato, and has been demonstrated to provide excellent protectant properties. However, despite its effectiveness, lesions appeared on 62.5% of inoculated plants due to the direct method of inoculation. However, the average length of developing lesions was much smaller than the untreated control (11.6 mm compared with 95.4mm at the last assessment) and this was statistically significant across all assessment dates. There was also only a slight increase in lesion size between the first and last assessments (5.8 mm to 11.6 mm).

Three products, Carial Star, AHDB9879 and AHDB9882, also significantly reduced stem lesion length compared with the untreated control but only up to the fourth disease assessment (third treatment application; 19/08/19).

Carial Star was granted full EAMU status in November 2018 for the control of late blight in tomato, and AHDB9879 and AHDB9882 both performed comparably. Carial Star should be considered the most appropriate industry standard in this trial, as this product provided improved control against the *P infestans* EU39_A1 strain, whilst Ranman Top did not. The modes of action of AHDB9879 (FRAC codes 22 and 4) and AHDB9882 (FRAC code 43 and 28) are different to the two registered products Ranman Top (FRAC code 21) and Carial Star (FRAC codes 3 and 40). Although these coded products did not outperform Carial Star, they

could be used to broaden the number of actives and modes of action available against *P. infestans* in an integrated control programme, improving resistance management.

Two other products AHDB9967 and AHDB9941 also significantly reduced stem lesion length, but on only two occasions. Interestingly AHDB9967 significantly reduced stem lesion length at assessments two and three, but not at assessment one although this was close to being significant. AHDB9967 is a biological product (plant extract) and although not fully effective in reducing stem lesion size at all assessment dates, it could be a useful component in organic production, and is the only biological product tested in this work which resulted in any control.

Delays in delivery of AHDB9941 meant that it could not be applied preventatively, but was applied curatively 24 hours after inoculation, immediately after the removal of the plastic film surrounding the inoculated wound. Despite this, AHDB9941 reduced lesion length compared with the untreated control at the first and second assessments. This product contained one of the same actives as in AHDB9882 (FRAC code 28), but with an additional active (FRAC code 33). AHDB9941 is used as a protectant fungicide with systemic action in multiple crops against oomycete pathogens including downy mildew and pythium. The efficacy of AHDB9882 may have been enhanced if this had been able to be applied preventatively. It is not possible to know if the different active in AHDB9982 enhanced its activity, or if the effects were due to the activity of the FRAC code 28 product alone.

The biological product AHDB9852 (a plant extract) provided no reduction in *P. infestans* incidence or severity compared with the untreated control. Although not statistically different, stem lesions were longer in plants receiving this treatment but further work would be needed to determine why this is the case. AHDB9852 is therefore not recommended for use in commercial tomato crops then as it could have a major impact on the severity of any future early *P. infestans* infections.

It should be noted that this trial used an artificial inoculation method which likely resulted in increased disease pressure compared to a naturally occurring inoculum source. This was done to ensure uniform infection developed in all plants, and to thoroughly assess the effectiveness of the test treatments. It is therefore possible that AHDB9841, and some of the other test products, may perform better in a commercial situation. Although all test products were applied at full rate, alone and generally more frequently than is permitted during commercial cropping it is noteworthy that no symptoms of phytotoxicity were seen on any plants, at any assessment date.

Some products tested in this work provided good control of *P. infestans* EU39_A1 strain in tomato. Effective products, such as AHDB9841, could be further assessed for their suitability for use in protected tomato as part of an integrated control program to maximize their role in fungicide resistance management.

Conclusions

- Only one product, AHDB9841, applied preventively and three times during cropping, significantly reduced the incidence of *P. infestans* following artificial inoculation.
- Seven of the products tested reduced disease severity (stem lesion length), on at least one assessment.
- Only AHDB841 reduced disease severity consistently at every assessment date.
- The industry standard Ranman Top, did not reduce disease severity compared to the untreated control at any assessment date, corresponding with reports of reduced sensitivity of this strain of *P. infestans* to Ranman Top.
- Two products, AHDB9879 and AHDB9882 performed comparably well to Carial Star, a product which was authorised for use against *P. infestans* in tomato in November 2018. These treatments have different modes of action to Carial Star and could be useful in resistance management.
- The biological product AHDB9967 significantly reduced disease severity at two assessments and could be used in organic production.
- Use of the biological product AHDB9852 appeared to increase stem lesion length compared with the untreated control.

- Although it was not possible to apply AHDB9941 preventively in this trial, the curative applications still resulted in significant reductions in stem lesion length compared with the untreated control at the first two disease assessments. AHDB9941 is a protectant and systemic fungicide and its effectiveness might be enhanced if it were to be applied preventatively.
- No visible symptoms of phytotoxicity developed on any treated plants, at any assessment date.

Acknowledgements

We would like to thank Chris Dyer for performing the statistical analysis, and AHDB Horticulture and participating crop protection companies for advice on product selection and use, and for supporting the SCEPTREplus program.

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Appendix

a. Crop diary

Species – Tomato
Cultivar – Elegance
Planted – July 2019

Cultivations, fertilisers, etc. – The trial was cultivated following normal commercial practices, minus CO_2 enrichment and the application of any fungicides not tested in this work. No chemical insecticides were applied, but predatory biocontrol agents were introduced every 14 days. Plants were de-leafed as necessary (consistently across all plants)

All fruit (red and green), were harvested on two occasions to prevent further bending of the support wires due to the weight of developing fruit.

Fertiliser inputs to trial area

Date	Product	Rate
Continuous fertigation using Dosatron: three times per day (7:00, 13:15 and 17:30) for 15 minutes	Solufeed Fleury	1 g per 1 L

Insecticide inputs to trial area

Date	Product	Rate
Every two weeks after plant arrival	*Phtyoseiulus persimilis* *Encarsia formosa*	10-15 sachets 2 bottles

b. Trial diary

Date	Event
15/07/2019	- Preparation of inoculum (20 plates of *P. infestans* EU39_A1 strain on pea:rye agar) - Slabs wetted up
17/07/2019	- Collection of the plants from the propagator
30/07/2019	- *Plants planted out onto slabs, strung up and fertigation Started
31/07/2019	- Removal of leaf leaving stub wound - Treatment application 1 (minus AHDB9941) - Preliminary assessment
01/08/2019	- Artificial inoculation or leaf stubs with EU39_A1 plugs
02/08/2019	- Removal of the plastic film surrounding inoculated leaf stubs - First application of AHDB9941 to inoculated stubs - Closure of the polytunnel doors and start of the biosecurity measures
05/08/2019	- Disease assessment 1 - Phytotoxicity assessment 1
09/08/2019	- Treatment application 2 (all treatments) - Disease assessment 2 - Phytotoxicity assessment 2
14/08/2019	- Disease assessment 3 - Phytotoxicity assessment 3
19/08/2019	- Treatment application 3 (all treatments) - Disease assessment 4 - Phytotoxicity assessment 4
29/08/2019	- Treatment application 4 (all treatments) - Disease assessment 5 - Phytotoxicity assessment 5

Date	Event
13/09/2019	- Disease assessment 6 - Phytotoxicity assessment 6

*Planting and trial commencement was delayed due to the extremely high temperatures experienced in mid/late July 2019, which would have negatively impacted the viability of *P. infestans*.

c. Experiment images

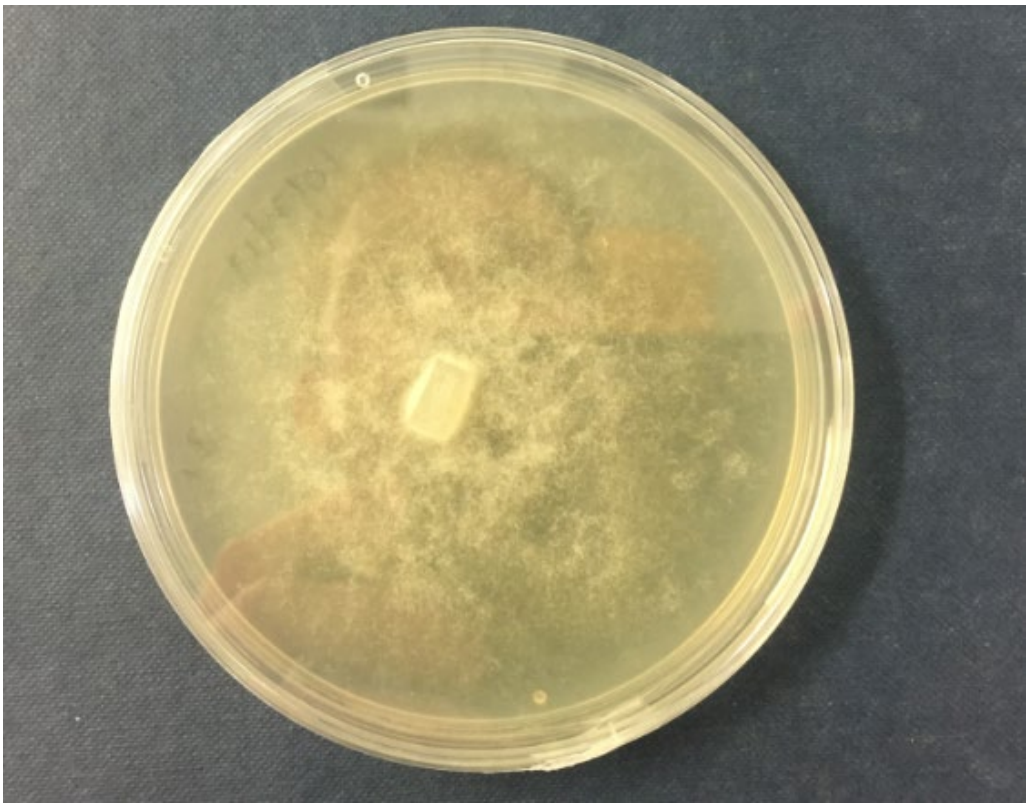


Plate of *P. infestans* EU39_A1 strain grown on pea:rye agar (July 2019).

Trial area before artificial inoculation (01 Aug 2019).

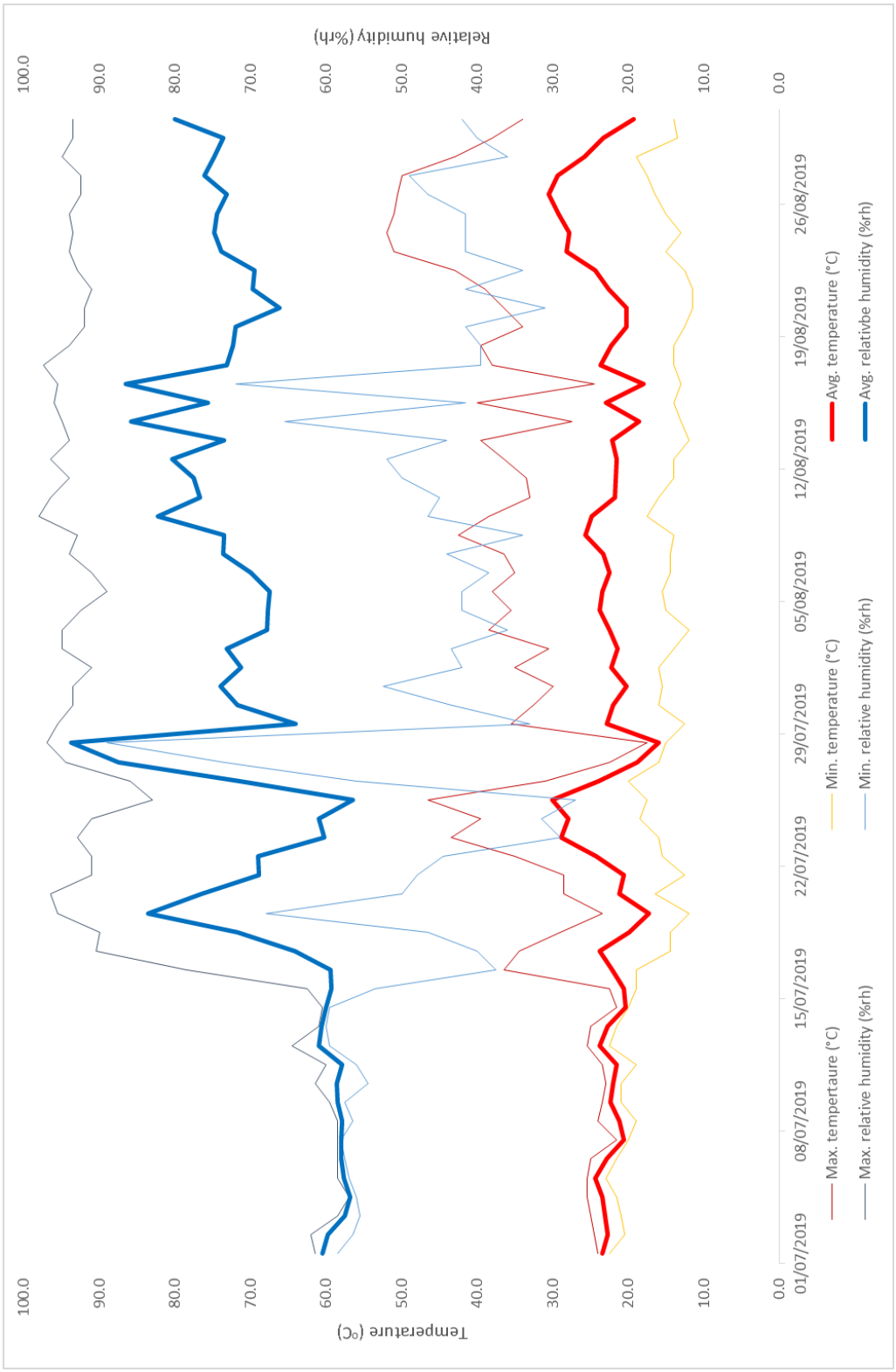
Leaf stub inoculated with a plug of *P. infestans* EU39_A1 strain, encased for 24 hours in cling film maintaining high humidity, aiding infection (01 Aug 2019)

Early stem lesion of *P. infestans* stem infecton at disease assessment one, 5 DA (05 Aug 2019).

Stem lesion of *P. infestans* infecton (08 Aug 2019).

Comparison of the lengths of infected stem lesions for all plants in plot 5 (untreated, left) and plot 20 (AHDB9841, right) at the final assessment (17 Sep 2019).

d. Climatological data during study period (01/07/19 – 30/08/19)



e. Raw data from assessments

P. infestans incidence (% of plants/plot infected)									
Block	**Plot**	**Treatment**	**31.07.19**	**05.08.19**	**09.08.19**	**14.08.19**	**19.08.19**	**29.08.19**	**13.09.19**
1	5	1	0.0	100.0	100.0	100.0	100.0	100.0	100.0
2	12	1	0.0	100.0	100.0	100.0	100.0	100.0	100.0
3	22	1	0.0	100.0	100.0	100.0	100.0	100.0	100.0
4	32	1	0.0	100.0	100.0	100.0	100.0	100.0	100.0
1	1	2	0.0	100.0	100.0	100.0	100.0	100.0	100.0
2	18	2	0.0	100.0	100.0	100.0	100.0	100.0	100.0
3	30	2	0.0	100.0	100.0	100.0	100.0	100.0	100.0
4	38	2	0.0	100.0	100.0	100.0	100.0	100.0	100.0
1	3	3	0.0	75.0	75.0	75.0	75.0	75.0	75.0
2	19	3	0.0	100.0	100.0	100.0	100.0	100.0	100.0
3	29	3	0.0	100.0	100.0	100.0	100.0	100.0	100.0
4	37	3	0.0	100.0	100.0	100.0	100.0	100.0	100.0
1	10	4	0.0	100.0	100.0	100.0	100.0	100.0	100.0
2	15	4	0.0	100.0	100.0	100.0	100.0	100.0	100.0
3	21	4	0.0	100.0	100.0	100.0	100.0	100.0	100.0
4	33	4	0.0	100.0	100.0	100.0	100.0	100.0	100.0
1	7	5	0.0	100.0	100.0	100.0	100.0	100.0	100.0
2	13	5	0.0	75.0	75.0	75.0	75.0	75.0	75.0
3	27	5	0.0	75.0	75.0	75.0	75.0	75.0	75.0
4	31	5	0.0	100.0	100.0	100.0	100.0	100.0	100.0
1	6	6	0.0	100.0	100.0	100.0	100.0	100.0	100.0
2	16	6	0.0	100.0	100.0	100.0	100.0	100.0	100.0
3	25	6	0.0	100.0	100.0	100.0	100.0	100.0	100.0
4	40	6	0.0	100.0	100.0	100.0	100.0	100.0	100.0
1	2	7	0.0	100.0	100.0	100.0	100.0	100.0	100.0
2	17	7	0.0	100.0	100.0	100.0	100.0	100.0	100.0
3	23	7	0.0	100.0	100.0	100.0	100.0	100.0	100.0
4	39	7	0.0	100.0	100.0	100.0	100.0	100.0	100.0
1	8	8	0.0	50.0	50.0	50.0	50.0	50.0	50.0
2	11	8	0.0	100.0	100.0	100.0	100.0	100.0	100.0
3	26	8	0.0	100.0	100.0	100.0	100.0	100.0	100.0
4	34	8	0.0	100.0	100.0	100.0	100.0	100.0	100.0
1	9	9	0.0	100.0	100.0	100.0	100.0	100.0	100.0
2	14	9	0.0	75.0	75.0	75.0	75.0	75.0	75.0
3	28	9	0.0	100.0	100.0	100.0	100.0	100.0	100.0
4	36	9	0.0	100.0	100.0	100.0	100.0	100.0	100.0
1	4	10	0.0	50.0	50.0	50.0	50.0	50.0	50.0
2	20	10	0.0	100.0	100.0	100.0	100.0	100.0	100.0
3	24	10	0.0	50.0	50.0	50.0	50.0	50.0	50.0
4	35	10	0.0	50.0	50.0	50.0	50.0	50.0	50.0

P. infestans severity - length of stem lesion (mm)									
Block	**Plot**	**Treatment**	**31.07.19**	**05.08.19**	**09.08.19**	**14.08.19**	**19.08.19**	**29.08.19**	**13.09.19**
1	1	2	0.0	19.8	52.3	105.3	146.3	162.0	170.5
1	2	7	0.0	14.3	48.5	96.5	128.8	157.8	176.3
1	3	3	0.0	6.3	15.3	35.5	53.0	66.3	68.8
1	4	10	0.0	4.5	5.3	5.3	5.3	6.0	6.3
1	5	1	0.0	13.8	44.8	77.0	113.5	117.8	125.3
1	6	6	0.0	15.8	43.8	83.8	110.0	138.3	142.8
1	7	5	0.0	8.8	13.5	20.5	52.5	63.5	65.5
1	8	8	0.0	3.3	3.3	3.3	4.0	4.0	4.5
1	9	9	0.0	9.8	22.8	40.5	43.5	49.8	58.5
1	10	4	0.0	9.3	14.8	31.5	55.8	72.3	82.3
2	11	8	0.0	17.0	24.0	27.0	43.3	51.3	59.8
2	12	1	0.0	16.0	37.5	46.8	55.5	66.5	68.3
2	13	5	0.0	5.3	5.3	5.3	5.5	5.5	8.3
2	14	9	0.0	8.8	17.5	26.3	39.0	43.5	79.3
2	15	4	0.0	11.3	10.0	22.5	37.5	45.8	48.5
2	16	6	0.0	19.0	58.0	106.0	149.5	182.3	192.8
2	17	7	0.0	11.5	30.3	43.8	59.5	60.5	67.3
2	18	2	0.0	12.5	22.3	26.8	42.0	49.8	61.3
2	19	3	0.0	9.0	17.0	27.3	67.0	79.8	89.8
2	20	10	0.0	8.5	7.8	8.0	9.0	9.5	9.5
3	21	4	0.0	11.8	13.0	15.0	23.3	30.0	37.0
3	22	1	0.0	13.0	26.0	31.0	40.0	44.3	44.8
3	23	7	0.0	9.8	20.8	29.5	49.0	62.8	66.3
3	24	10	0.0	6.0	10.0	16.3	21.3	21.3	26.5
3	25	6	0.0	10.3	25.0	41.0	65.0	69.8	82.5
3	26	8	0.0	16.0	29.3	33.8	57.0	65.0	78.8
3	27	5	0.0	6.0	7.0	7.5	10.0	11.3	16.8
3	28	9	0.0	10.0	18.8	25.0	28.0	42.0	47.8
3	29	3	0.0	9.3	6.8	13.3	17.0	21.5	22.5
3	30	2	0.0	10.0	9.8	10.0	26.0	35.3	38.8
4	31	5	0.0	8.0	29.3	58.8	88.3	113.0	115.8
4	32	1	0.0	31.3	65.3	92.3	125.8	135.0	143.3
4	33	4	0.0	9.5	13.8	22.3	31.3	41.0	49.0
4	34	8	0.0	10.8	21.0	30.0	62.8	80.5	92.0
4	35	10	0.0	4.0	3.8	3.8	3.8	3.8	4.0
4	36	9	0.0	7.0	21.3	35.0	44.0	47.5	55.5
4	37	3	0.0	8.3	20.3	22.8	29.0	32.8	40.3
4	38	2	0.0	8.3	27.5	46.3	62.3	68.5	74.0
4	39	7	0.0	7.8	20.5	36.0	56.8	63.8	65.8
4	40	6	0.0	19.3	44.0	80.8	107.8	129.8	138.3

f. Trial design

	AHDB9882	Control	AHDB9879	AHDB9967	AHDB9841	AHDB9941	Carial Star	Ranman Top	AHDB9959	AHDB9852	
Plot	31	32	33	34	35	36	37	38	39	40	Block 4
	AHDB9879	Control	AHDB9959	AHDB9841	AHDB9852	AHDB9967	AHDB9882	AHDB9941	Carial Star	Ranman Top	
Plot	21	22	23	24	25	26	27	28	29	30	Block 3
	AHDB9967	Control	AHDB9882	AHDB9941	AHDB9879	AHDB9852	AHDB9959	Ranman Top	Carial Star	AHDB9841	
Plot	11	12	13	14	15	16	17	18	19	20	Block 2
	Ranman Top	AHDB9959	Carial Star	AHDB9841	Control	AHDB9852	AHDB9882	AHDB9967	AHDB9941	AHDB9879	
Plot	1	2	3	4	5	6	7	8	9	10	Block 1

g. Water analysis results (sample collected and sent on 15-Aug-2019)

Determinand	Value	Units
pH	7.3	
Nitrate N	8.9	mg/l
Sulphate as SO_4	31.2	mg/l
Boron	0.03	mg/l
Copper	<0.01	mg/l
Manganese	<0.01	mg/l
Zinc	<0.01	mg/l
Iron	<0.01	mg/l
Conductivity	607	uS/cm
Chloride	33.4	mg/l
Phosphorus as P	1.0	mg/l
Potassium	2.4	mg/l
Magnesium	4.22	mg/l
Calcium	118.4	mg/l
Sodium	13.6	mg/l

h. ORETO certification

Certificate of

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

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Stored Crops
Biologicals and Semiochemicals

Date of issue: 1 June 2018
Effective date: 18 March 2018
Expiry date: 17 March 2023

Signature Alison Richardson
Authorised signatory

Certification Number
ORETO 409

HSE
Chemicals Regulation Division

Department of Agriculture and Rural Development