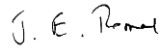
SCEPTREPLUS

Final Trial Report

Trial code:	SP65
Title:	Novel approaches for bacterial disease control in outdoor field vegetables
Crop	Outdoor cucurbits – autumn squash. The report may also be relevant to bacterial diseases on other outdoor field vegetables and outdoor ornamentals
Target	*Pseudomonas syringae* pv*. lachrymans*
Lead researcher:	Jane Thomas
Organisation:	NIAB
Period:	May 2020 -January 2021
Report date:	Dec 2021
Report author:	Jane Thomas
ORETO Number: (certificate should be attached)	ORETO 397

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 December 2021



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Trial Summary

The loss of copper-based products for the control of bacterial diseases in outdoor vegetables and ornamental crops has meant that new approaches are needed to reduce symptoms induced by bacterial infections to acceptable levels. At the same time, growing conditions for certain crops are likely to become more conducive to bacterial infections. In this investigation, the effects of alternative products for the control of angular bacterial leaf spot of field-grown autumn squash were evaluated. The pathosystem may serve as an indicator for the control of other bacterial diseases on outdoor crops, but is also an emerging disease of importance in UK outdoor cucurbits. Warmer summer conditions, and increased use of irrigation, are both factors which are likely to lead to increased prevalence of blemishes on fruit, leading to loss of quality. The causal organism (*Pseudomonas syringae* pv *lachrymas*, *Psl*) is seed-borne, and can infect plants during indoor raising, providing inoculum for later transfer to leaves and fruit.

Methods

Plants of the autumn squash variety Windsor were reared in a glasshouse during April 2020 and then transplanted into the field in May. Plants were allowed to develop until fruits were forming and then treated with experimental products. After 48 h they were inoculated with a suspension of bacteria derived from a culture of *Pseudomonas syringae* pv. *lachrymans*. A second application of experimental products was made eight days after the first. Fruits were scored for typical lesions in the field in October. A sub-sample of fruits was also harvested and assessed for disease and then held at ambient in an unheated barn area before a final assessment eighteen days after harvest.

Squash seed of the same stock of Windsor was also planted in seed trays under glass in December 2020 and grown to the first two true leaves stage. The plants were inoculated with the same strain of *P.syringae* pv. *lachrymans* and then treated with experimental products after 24 h. Disease severity on leaves was then assessed 14 and 30 days after treatment application.

Two rates of a botanical extract, three biological control products, a sulphur-based product and a plant oil extract were compared with untreated in two phases a) field grown fruits, and b) glasshouse grown plants. In each case, test material was inoculated with an isolate of *Psl*, originally obtained from cucumber in the UK. The test products are all available in the UK, registered on other crops. As copper-based products are no longer available for use, it was decided not to use any as an experimental control.

Results

Results from the fruits are presented as mean values with statistical summary in the tables below. Field plot % scores are visual estimates of the visible area of fruits affected by spotting. Field plot 0-5 scores are visual estimates of the severity of spotting, based on a score of 1 being <1% area affected, and 5 being > 10% area affected. The sampled sub-sets were scored on the same 0-5 scale, and consisted of fruits removed from the field so that the whole area could be seen. In the greenhouse trial, leaves were first assessed on a 0-7 severity scale where 1 was <5 % leaf area infected and 7 was >50% infected. The subsequent score was based on visual estimate of % leaf area infected.

Symptoms of *Psl* appeared one week after inoculation on fruits as small watersoaked spots with white exudate in the centre on untreated plots. Larger spreading blotches were also seen. On foliage in the glasshouse, typical white angular spots were seen, coalescing into larger brown areas and becoming “shot-holed”. None of the products tested showed any evidence of phytotoxicity. In the field trial, there was evidence of partial control initially, and all the tested products showed significant reduction in disease compared to the untreated control, but these significant effects declined both in the field and in a harvested sub-set of fruits. Orocide still resulted in a significant reduction in disease compared to the untreated in the first score of the sampled sub-set, and the effects of AHDB9885 and Serenade were significant at the second scoring time point. Clear and significant effects were seen for most products in the greenhouse trial, and hence soon after infection may be the optimum application timing to control the disease. All products gave significant reductions in disease at the first assessment with the exception of Orocide and all gave significant reduction at the second assessment, with the high rate of AHDB9885, Botector and Serenade ASO showing the largest symptom reduction compared to the untreated foliage. Effects of all products were generally poorer on fruit than on foliage. However, some could still prove valuable alternatives to copper based products.

1. Field test

	Field plot score (% area infected) 9/10/20	**Field plot score (0-5) 30/10/20**	**Sampled sub-set (0-5) 13/10/20**	**Sampled sub-set (0-5) 31/10/20**
Treatment				
Untreated	5.43	2.17	3.28	3.98
AHDB9885 low rate	2.08	2.17	2.49	3.48
AHDB9885 high rate	2.18	2.50	2.49	3.31
Botector	2.07	1.25	3.03	3.64
Kumulus DF	2.30	2.00	2.58	3.71
Orocide	1.67	1.50	2.33	3.61
Serenade ASO	2.07	2.00	2.65	3.33
Romeo	3.12	2.67	2.71	3.73
	Not significantly different from untreated control (p>0.05)			
	Significantly different from untreated control (p<0.05)			

* 5=most severe

2. Glasshouse test

	Foliage score 0-7*	Foliage score % leaf area infected
Treatment		
Untreated	2.8	66.21
AHDB9885 low rate	1.9	41.05
AHDB9885 high rate	2.0	31.76
Botector	1.7	29.46
Kumulus DF	2.1	52.20
Orocide	2.4	57.16
Serenade ASO	1.5	34.25
Romeo	2.0	42.24
	Not significantly different from untreated control (p>0.05)	
	Significantly different from untreated control (p<0.05)	

*7=most severe

Conclusions

Inoculation with *Psl* produced typical disease symptoms on fruits in the field and on foliage in the glasshouse. Some tested products significantly reduced symptoms in both environments. The high rate of AHDB9885, a botanical extract, and Serenade ASO, a bacterial biocontrol product, resulted in the most persistent effects on fruit in the field. All products were effective to varying degrees on foliage.

Take home message:

Several products tested merit further investigation for the control of *Psl* on cucurbits, including AHDB9885, Botector, and Serenade ASO, and potentially for bacterial diseases of other field grown crops

Objectives

1. To establish an effective test system for antibacterial products for field grown crops using angular leaf spot of outdoor cucurbits as an exemplar.
2. To generate efficacy data of potential products for copper replacement
3. To evaluate products 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(4)	Design and analysis of efficacy evaluation trials	None
PP 1/135(4)	Phytotoxicity assessment	None
PP 1/181(4)	Conduct and reporting of efficacy evaluation trials including GEP	None

There are no EPPO guidelines for *Psl* on cucurbits. Six replicates were used for both the field assessment on fruits and the glasshouse assessment on foliage as usual practice for biological products.

Test site - field

Item	**Details**
Location address	NIAB, Park Farm, Villa Road, Histon, Cambridge, CB24 9NZ
Crop	Autumn squash
Cultivar	Windsor
Soil or substrate type	Soil – Clay loam
Agronomic practice	Irrigation as required during field establishment. Planted through Mypex for weed control. Prilled N applied before Mypex cover, and slug pellets over Mypex. Rabbit fencing and bird scarers installed after transplanting
Prior history of site	Field beans

Trial design

Item	**Details**
Trial design:	Randomized block
Number of replicates:	6
Row spacing:	3
Plot size: (w x l)	1m x 2m
Plot size: (m^2)	2
Number of plants per plot:	3 in centre test row
Leaf Wall Area calculations	Not applicable

Trial maintenance and inoculation methods

Field trial

Untreated seeds of autumn squash cv. Windsor were planted in a glasshouse on 27/04/20 and grown for 4 weeks before hardening off in an outdoor standing area for 7 days. Plants were than transplanted to the field on 28/05/20 through Mypex to suppress weeds. Discard plots of butternut squash (cv. Pacific Royale) were planted between each run of test plots. Plants were irrigated at planting and then at intervals to ensure survival and growth. Gaps were filled as necessary.

A UK isolate of *Pseudomonas syringae* pv *lachrymas*, (*Psl*) from cucumber was obtained from the NCPPB, accession number 3544 and cultured on nutrient agar plates. Bacterial suspensions of *Psl* were prepared by flooding plates in sterile distilled water and agitating the solution. Optical density was measured at 600nm with a spectrophotometer and diluted to achieve a suspension equivalent to 1 x 10^8 colony forming units ml^{-1}. 50 ml of this inoculum was applied to the surface of the central row of squash fruits on 24/09/20 and again on 02/10/20. Test products were applied on 22/09/20 and 30/09/20 when foliage had partially collapsed and fruits were exposed to the spray.

Fruits were scored for the severity of angular leaf spot disease on two occasions in the field using a visual assessment of % area infected on fruits (09/10/20) and then on an overall plot basis on remaining fruits on a 0-5 scale, where 5 is most severe, on 30/10/20. Scale points are described in the assessment details section. A sub-sample of fruits was harvested on 12/10/20 consisting of 3 or 4 fruits from the centre of each plot and avoiding any that may have been exposed to spray from neighbouring plots. Each fruit was assessed separately using the 0-5 scale the day after harvest, and again eighteen days later on 31/10/20. Fruits were stored in an unheated barn during this period.

Glasshouse trial

Untreated seeds of autumn squash cv. Windsor were germinated in seed trays (30 cm x 20 cm; 30 seeds per tray) in a growth room set at 25°C (day), 18°C night and a 16 h day to promote germination and early growth before being moved to a glasshouse in January 2021, with temperatures between 20-22°C day and 15-18°C night. Natural daylight was supplemented by sodium lights to give a 16 h day. Plants were inoculated when 2 true leaves were fully expanded with a *Psl* bacterial suspension at 5 x10^8 cfu ml^{-1} prepared as for the field trial, and covered with polythene sheets for 24h. Six replicate trays were used per treatment in a randomized block design. Leaves were assessed for angular leaf spot disease 14 days after treatment application. Plants were then re-inoculated 19 days after the first treatment with a *Psl* suspension of 6 x 10^8 cfu ml^{-1}, and scored for disease again after a further sixteen days, but no further treatment applications were made. The first assessment used a 0-7 scale, where 7 is most severe, and the second scored % leaf area infected. Each plant was assessed separately on upper and lower leaves. The scale points are described in the assessment details section.

Treatment details – Field trial and glasshouse trial

AHDB Code	Active substance	Product name/ manufacturers code	Formulation batch number	Content of active substance in product	Formulation type	Adjuvant
Untreated						
AHDB9885 low rate	N/D	N/D	11001	N/D	N/D	N/A
AHDB9885 high rate	N/D	N/D	11001	N/D	N/D	N/A
-	Aureobasidium pullalans 14940 and 14941	Botector	2021/0018	500g/kg	DG	N/A
-	Sulphur	Kumulus DF DF		80% w/w sulphur	DG	N/A
-	Sweet orange extract	Orocide		6%		
-	Bacillus amyloliquifasciens	Serenade ASO		1015.1 g/l	SC	N/A
-	Saccharomyces cerevisiae cell walls	Romeo		10% Cerevisane	WP	N/A

Application schedule – field trial

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	Untreated			
2	AHDB9885	N/D	N/D	A, B
3	AHDB9885	N/D	N/D	A, B
4	Botector	500 g/ha	1 kg/ha	A, B
5	Kumulus DF	8 kg/ha	10 kg/ha	A, B
6	Orocide	36 ml/ha	600 ml/100 l water	A, B
7	Serenade ASO	8120.8 g/ha	8 l/ha	A, B
8	Romeo	25 g/ha	0.25 kg/ha	A, B

Application details – field trial

	Application A	Application B
Application date	22/09/20	30/09/20
Time of day	14.45-15.45	12.00-13.00
Crop growth stage (Max, min average BBCH)	702	703
Crop height (cm)	40	40
Crop coverage (%)	Fruits only	Fruits only
Application Method	Spray	spray
Application Placement	Fruit	fruit
Application equipment	EP-100	EP-100
Nozzle pressure	2.5 bar	2.5 bar
Nozzle type	Induction 110	Induction 110

Nozzle size	N/A	N/A
Application water volume/ha	300 l	300 l
Temperature of air - shade (°C)	24.8	12.7
Relative humidity (%)	39.1	82.3
Wind speed range (m/s)	1.79	0.45
Dew presence (Y/N)	N/A	N/A
Temperature of soil - 2-5 cm (°C)	N/A	N/A
Wetness of soil - 2-5 cm	N/A	N/A
Cloud cover (%)	10	30

Application details – glasshouse trial

Application details for the glasshouse experiment followed the same rates as the field, adjusted for the combined area of six seed trays (35 cm x 20 cm) per treatment. Application volume was increased to achieve leaf coverage. Products were applied 24h after inoculation with *Psl* as a single application, carried out on 14/01/21 using small hand held sprayers in a covered area and plants allowed to stand for 4-5 h before being returned to the glasshouse.

Application details – glasshouse trial

	Application A
Application date	14/01/21
Time of day	11.00-15.00
Crop growth stage (Max, min average BBCH)	102
Crop height (cm)	10
Crop coverage (%)	100% leaves
Application Method	Spray
Application Placement	Leaf
Application equipment	Hand held Hozelock
Nozzle pressure	N/A
Nozzle type	Cone
Nozzle size	N/A
Application water volume/ha	600l
Temperature of air - shade (°C)	20
Relative humidity (%)	N/A
Wind speed range (m/s)	N/A
Dew presence (Y/N)	N/A
Temperature of soil - 2-5 cm (°C)	N/A
Wetness of soil - 2-5 cm	N/A
Cloud cover (%)	N/A

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
Angular leaf spot	*Pseudomonas syringae* pv *lachrymans*	PSDMLA	none visible (field)	moderate	moderate
Angular leaf spot	*Pseudomonas syringae* pv *lachrymans*	PSDMLA	none visible (glasshouse)	moderate	High
Powdery mildew	*Podosphaeria xanthii*	PODOXA	Low (field)	Low	Low

Assessment details

Evaluation date	**Days after sowing**	**Crop Growth Stage (BBCH)**	**Evaluation type (efficacy, phytotox)**	**Assessment**	**Assessment type**
25/09/20	119	702	phyotoxicity	Foliage and fruit	
09/10/20	133	703	Phytotoxicity	Foliage and fruit	
30/10/20	154	704	Phytotoxicity	Foliage and fruit	
09/10/20	133	703	Efficacy	In field fruit score	% fruit area
30/10/20	154	704	Efficacy	In field fruit score	0-5
13/10/20	137	N/A	Efficacy	Sampled fruit score	0-5
31/10/20	155	N/A	Efficacy	Sampled fruit score	0-5
21/01/21	52	12	Phytotoxicity	Foliage in glasshouse	
28/01/21	59	12	Efficacy	Foliage in glasshouse	0-7
18/02/21	80	13	Efficacy	Foliage in glasshouse	% leaf area

Phytotoxicity symptoms were checked at every trial visit, and formally noted on the dates above.

Disease severity scales for the field trial were either a) a visual assessment of % fruit area infected per plot or b) a 0-5 estimation of infection levels as shown in the table below. The same scale was used for sub-samples of fruit removed for disease assessment

Scale point	Description
0	Healthy, no visible sign of infection
1	scattered lesions< 1% fruit area infected
2	scattered lesions>1% <5% fruit area infected
3	scattered lesions, 5% fruit area infected
4	widespread lesions 6-10% fruit area infected
5	spreading watersoaked lesions > 10% fruit area infected

For the first assessment of foliar infection in the glasshouse, the following disease severity scale was used:

Scale point	Description
0	Healthy, no visible sign of infection
1	<5 % leaf area infected, scattered lesions
2	between 5-10% leaf area infected
3	11-20% leaf area infected, merging lesions
4	21-30% leaf area infected
5	31-40% leaf area infected
6	41-50% leaf area infected
7	>50% leaf area infected, large merged lesions

A % leaf area visual assessment was used for the subsequent score.

Statistical analysis

Statistical analysis was carried out by NIAB statistics group using ANOVA in Genstat. For the glasshouse experiment, all individually assessed leaves were included as separate data points in the analysis.

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Phytotoxicity

No typical phytotoxicity such as scorching, chlorosis, necrosis or malformation was recorded for any treatment. The sulphur product left a white deposit on foliage, but no adverse plant reactions to this were observed and under field conditions it disappeared rapidly.

Efficacy

The inoculation technique produced moderately high disease pressure in the field trial. There was a relatively high degree of variation in disease levels between individual fruits and statistically significant differences between treatments were limited. In the glasshouse experiment, between replicate (seed tray) variation for treatments was very much less, and clear and significant differences were seen between treatments.

Results and summary statistics are shown in Table 1 (fruits in field and after harvest) and Table 2 (foliage in glasshouse), and graphically in Figure 1 (first field assessment) and Figure 2 (second foliage score) respectively for products in order of increasing severity. Percentage control for these two assessments compared to untreated is shown in Table 3.

Table 1 Field and harvest sub-sample disease assessments of fruit infection with *Psl* for different crop protection products

	Field plot score (% area infected) 9/10/20	**Field plot score (0-5) 30/10/20**	**Sampled sub-set (0-5) 13/10/20**	**Sampled sub-set (0-5) 31/10/20**
Treatment				
Untreated	5.43	2.17	3.28	3.98
AHDB9885 low rate	2.08	2.17	2.49	3.48
AHDB9885 high rate	2.18	2.50	2.49	3.31
Botector	2.07	1.25	3.03	3.64
Kumulus DF	2.30	2.00	2.58	3.71
Orocide	1.67	1.50	2.33	3.61
Serenade ASO	2.07	2.00	2.65	3.33
Romeo	3.12	2.67	2.71	3.73
Trial Mean	2.61	2.03	2.70	3.60
Standard error	0.817	0.646	0.348	0.293
LSD	1.634	1.293	0.697	0.586
P value	0.001	0.408 (NS)	0.05	0.05
	Significantly less than untreated control			

Table 2 Assessments of foliage infected with *Psl* in glasshouse experiment for different crop protection products

	Foliage score 0-7	**Foliage score % leaf area infected**
Treatment		
Untreated	2.8	66.21
AHDB9885 low rate	1.9	41.05
AHDB9885 high rate	2.0	31.76
Botector	1.7	29.46
Kumulus DF	2.1	52.20
Orocide	2.4	57.16
Serenade ASO	1.5	34.25
Romeo	2.0	42.24
Trial mean	2.1	44.29
Standard error	0.230	2.165
LSD	0.440	4.243
P value	<0.05	<0.05
	significantly less than untreated control	

All products significantly reduced the % of fruit area infected by *Psl* in the field at the first assessment date (09/10/20), but this reduction did not persist until the second assessment date three weeks later (Table1). When fruits were harvested and assessed indoors on 13/10/20 and 31/10/20, all products still resulted in a reduction in levels of *Psl*, but this was only significant in the case of Orocide. After ambient storage of fruits for a further eighteen days, reductions in disease were still apparent, but the differences from untreated were much smaller than the first field score and only significant for the higher rate of AHDB9885 and Serenade ASO.

In the glasshouse trial (Table 2), all products except Orocide significantly reduced disease symptoms at the first assessment (28/01/21) and all products resulted in statistically significant reductions at the second assessment (18/02/21) though for some products the reduction compared to untreated was relatively small. The greatest degree of control was seen for the higher rate of AHDB9885, Botector and Serenade ASO.

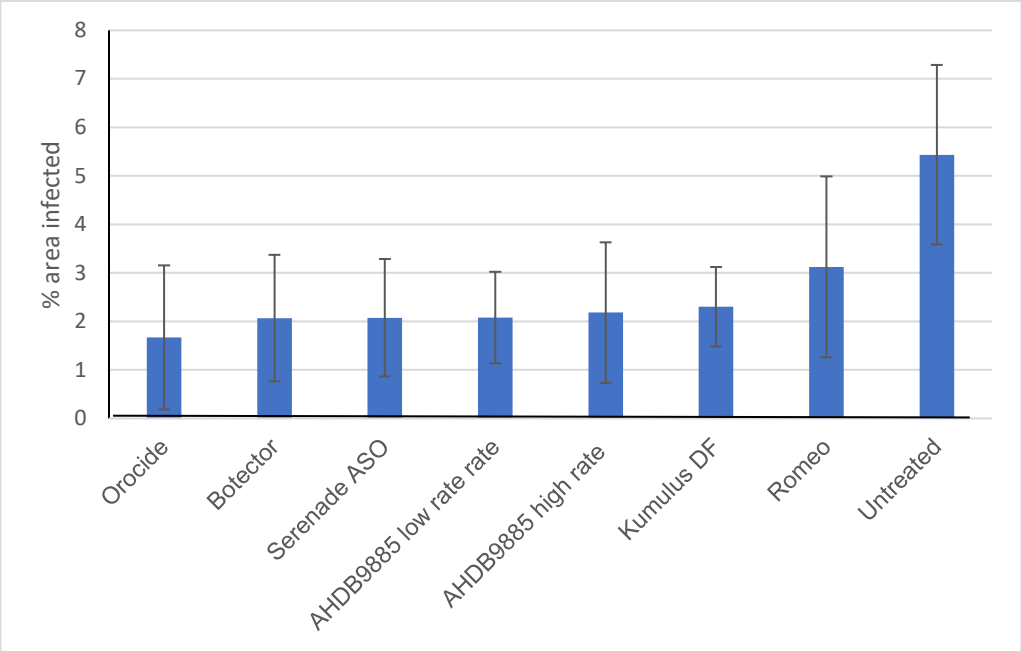


Figure 1 Percentage fruit area with *Psl* symptoms, at the first field assessment (09/10/20) for different crop protection products

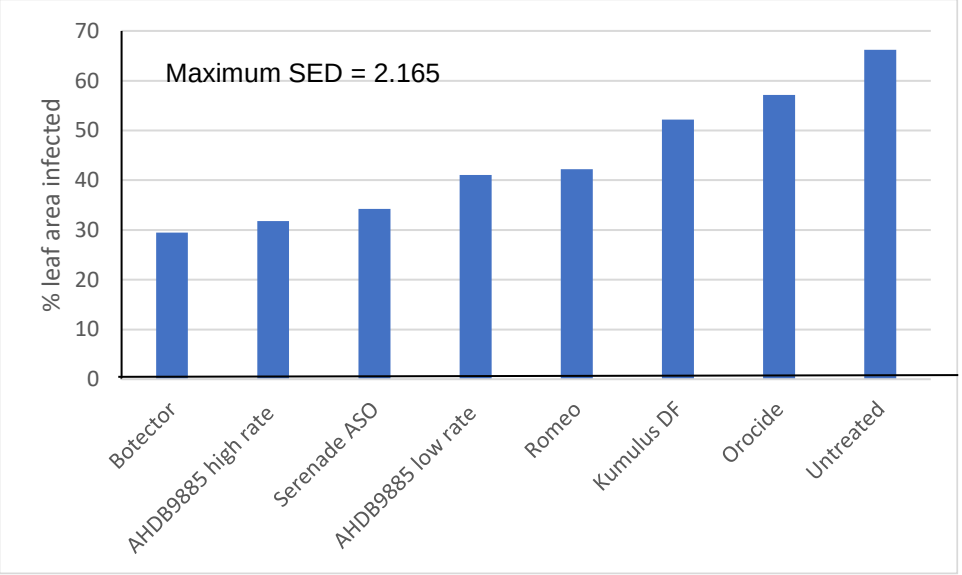


Figure 2 Percentage leaf area infected with *Psl* symptoms, at the second glasshouse assessment ((18/02/21) for different crop protection products

Several products achieved relatively high percentage control of disease symptoms on fruit in the field and glasshouse (Table 3). Orocide was noticeably more effective in the field than on foliage in the glasshouse. The high rate of AHDB9885, Botector, and Serenade ASO were the most consistent products over the two assessments.

Table 3 Percentage control compared to untreated for different crop protection products in the first field assessment on fruits, and the second glasshouse assessment on foliage

Treatment	Fruit assessment field	Foliage assessment glasshouse
AHDB9885 low rate	61.7	38.0
AHDB9885 high rate	59.8	52.0
Botector	61.9	55.5
Kumulus DF	57.6	21.2
Orocide	69.2	13.7
Serenade ASO	61.9	48.3
Romeo	42.5	36.2

Percent reduction was calculated as:

$$\frac{\text{Untreated score} - \text{treatment score}}{\text{Untreated score}} \times 100$$

Discussion

The results show that all of the tested products initially significantly reduced bacterial disease symptoms on fruit in the field to varying extents. Though the observed effects declined in the field and after the fruits were harvested, the higher rate of one botanical extract product (AHDB9885), and the biological product Serenade ASO still gave significant if small reductions in disease symptoms. These two products, together with the biological product Botector, also performed strongly in the glasshouse test on foliage. Combinations of products were not tested, but the use of combined botanical extract and biological products in integrated programmes merits further investigation. Use of biologicals during the plant raising stage when there is opportunity to colonise leaves in the absence of outdoor weather effects may be a promising approach to limit development from any seed-borne infection. Botanical extracts could then be used in subsequent treatments outdoors to maintain downward pressure on the development of pathogenic bacteria.

Originally, seed lots that agents thought may be infected with *Psl* were sourced for the trial, but of those provided (cvs Windsor, Red October, Autumn Gold and Pacific Royale) no symptoms of *Psl* were seen in glasshouse raised seedlings, and an isolate of *Psl* was therefore sourced from the NCPPB for the experiments using artificial inoculation. In the field trial, treatments were not applied until the fruiting growth stages as application to foliage at regular intervals throughout the field trial would have resulted in permitted spray numbers being exceeded for some products. Therefore, a glasshouse test was conducted separately to evaluate the efficacy of products under plant raising conditions. Since this stage is a likely source of infection

in the field if seed is infected, the data obtained provide important information for alternatives to copper based sprays. The field trial involved treating fruits which were then inoculated, but in the glasshouse test, leaves were inoculated before treatment. This was done after consultation with an industry advisor, as certain biological products may operate more effectively when host plants have already been challenged with a pathogen.

Part of the rationale for selection of an outdoor cucurbit for the trial protocol was to use the crop as an exemplar for other outdoor crops affected by bacterial pathogens. Though the test has generated useful data, there were difficulties with assessments in the field where the fruit area was not completely visible due to growth habit. In addition, fruits were prone to extend some distance from the mother plant. This restricted the number of fruits which could be assessed, since some may have been exposed to neighbouring plot sprays, despite the use of discard rows. Selection of a more upright, bushy variety type, with fruits held closer to the mother plant would avoid this in any future trials. Nevertheless, it was possible to obtain useful data from field assessments, and from fruit picked and assessed indoors, with the latter element enabling disease scores to be made of the whole fruit area, with none hidden by position on the ground. Typical symptoms of *Psl* were recorded, with small water soaked spots and larger water soaked blotches evident. Bacteria were also recovered from affected areas, showing fluorescence on Kings Medium B confirming they were Psudomonas spp. No fungal contaminants were isolated from the lesions.

Application in the field and glasshouse was successful for all products, with no spray blocking problems encountered, though Botector required significant agitation to disperse into solution.

The glasshouse test gave the clearest discrimination between product efficacy. It is notable that product activity overall persisted when a second inoculation was made, with no further treatments being applied while in the field, initial positive effects of products declined over time. Crop microclimate, rainfall, and interactions of the biological products with these factors and fruit surface microflora is likely to create variable outcomes. Specific investigations of promising biological products will be necessary to understand the conditions where the most consistent results are likely to be obtained.

Conclusions

<u>Field trial</u>

- Disease developed to a moderately high level in untreated plots
- All products except one showed an initial significant reduction in *Psl* symptoms
- Persistence of disease reduction effects was variable, and on harvested fruit only the higher rate of AHDB9885 and Serenade ASO showed significant reduction in disease
- Field efficacy of most products, while initially promising, requires further investigation to understand activity and optimize efficacy
- Outdoor cucurbits and *Psl* could be a useful model for studying bacterial diseases of field grown crops, but careful selection of variety type and growth habit is necessary

Glasshouse trial

- All products were effective in reducing foliar symptoms of *Psl*.
- The high rate of AHDB9885, Botector and Serenade ASO were most effective, and would merit further investigation.
- Applications of products made during the raising stage, when symptoms of *Psl* may spread rapidly from small numbers of infected seeds, could offer protection after transplanting and thus suppress development of symptoms on fruit

No phytotoxicity was observed with any product in either test.

Acknowledgements

Funding from AHDB SceptrePlus is gratefully acknowledged. Thanks are also due to manufacturers for supply of products, industry representatives for helpful discussions, and to breeders for supply of seed lots.

Appendix A

Crop diary - field

Date	Event
27/04/20	Planting in modules under glass
18/05/20	Prilled N applied to trial area
19/05/20	Mypex laid
21/05/20	Plants moved to stand out area
27/05/20	Holes made. Bird scarers and rabbit fencing
28/05/20	Transplanted through Mypex
28/05/20	30 ml irrigation, and subsequently as needed
29/05/20	Slug pellets applied to trial area
29/05/20	Gaps filled, and subsequently as necessary

Appendix B Trial diary

Field

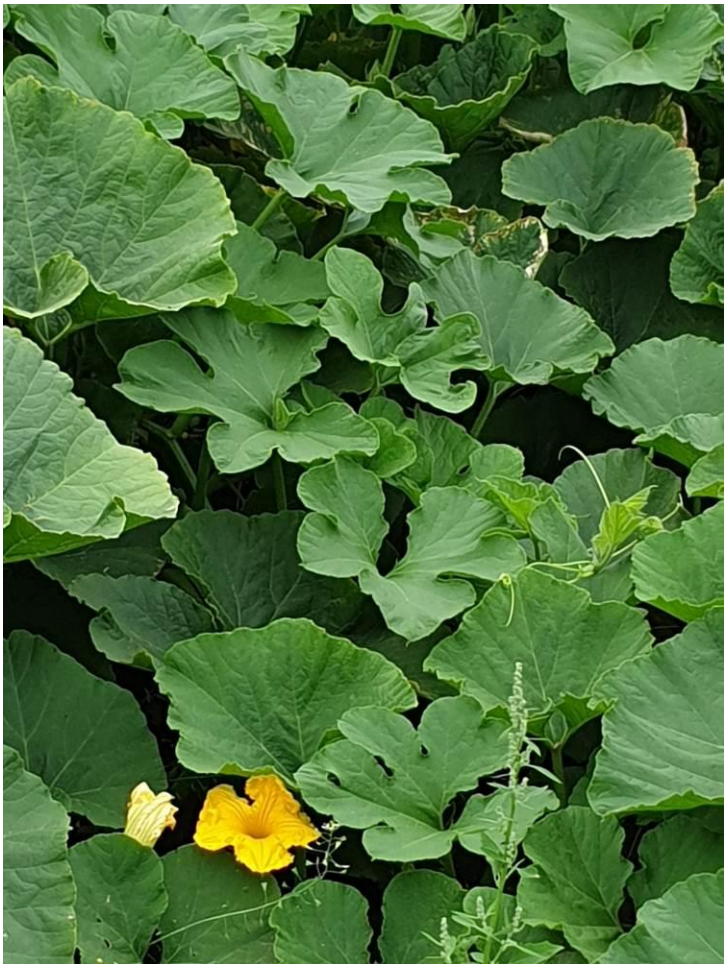
Date	Event
08/09/20 – 15/09/20	Increase *Psl* on nutrient agar
22/09/20	Treatments applied
24/09/20	Prepare inoculum and apply
30/09/20	Treatments re-applied
02/10/20	Prepare inoculum and apply

Glasshouse

Date	Event
01/12/20	Seed trays planted in growth room
13/12/20	Seed trays moved to glasshouse
05/01/21	Increase *Psl* on nutrient agar
13/01/21	Inoculation of leaves with *Psl*
14/01/21	Treatment with all products
02/02/21	Repeat inoculation with *Psl*

Appendix C Trial photographs

Plot view 07/07/20



Plot foliage, 14/08/20

Spotting and blotching, 02/10/20 on untreated plots, 8 days after inoculation

Spots with white central exudate 07/10/20

White papery lesions and shot-holes, glasshouse test, 26/01/21

Appendix D Climatalogical data

Taken from NIAB Cambridge met. station during field trial period

	Max°C	Min°C	Rain (mm)
June	21.2	10.6	47.2
July	21.9	12.4	50.6
August	23.9	14.3	104.0
September	20.1	9.8	51.4
October	14.1	8.2	116.4

Appendix E Raw data

Field score on fruits, % area infected 09/10/20

Treatment	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
Untreated	3.00	4.80	6.83	3.79	7.67	6.50
AHDB9885 low rate	1.00	3.50	2.40	1.53	1.38	2.67
AHDB9885 high rate	1.00	2.21	5.00	1.75	1.25	1.88
Botector	1.25	2.40	4.00	1.70	0.25	2.80
Kumulus DF	2.50	2.50	1.10	1.70	3.50	2.50
Orocide	4.20	0.88	2.00	0.75	0.00	2.17
Serenade ASO	2.83	1.38	1.86	1.88	0.50	4.00
Romeo	0.60	1.25	3.25	4.79	3.60	5.25

Field score on fruits,0-5 scale 30/10/20

Treatment	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
Untreated	3	1	4	1	3	1
AHDB9885 low rate	1	4	3	1	1	3
AHDB9885 high rate	2	4.5	3	1.5	3	1
Botector	0.5	2	2	1	1	1
Kumulus DF	1	2	2	2	3	2
Orocide	1	2	2	1.5	0.5	2
Serenade ASO	1.5	3.5	1	3	1	2
Romeo	1	1	2	5	3	4

Assessment on harvested fruit sample 0-5 scale 13/10/20

Treatment	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
Untreated	2	5	3.2	3.5	3	2.5
AHDB9885 low rate	2.3	3	2	2	2.3	2.9
AHDB9885 high rate	2.5	2.1	3.3	2.5	2.3	2.5
Botector	3.2	2.6	4.5	2.8	3	3
Kumulus DF	2.4	2.5	2.3	2.5	2.75	2.9
Orocide	4	3	1.5	2.1	3	1
Serenade ASO	2.3	2.5	3.1	2.3	1.8	3.1
Romeo	2.5	2.3	3	2.3	2.9	3.5

Assessment on harvested fruit sample 0-5 scale 30/10/20

Treatment	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
Untreated	3.8	5	4.2	4.3	3.8	3.5
AHDB9885 low rate	3.8	4	2.6	2.5	4	3.6
AHDB9885 high rate	3.5	3.1	4	3	3	3
Botector	3.7	3	5	4	3.5	4
Kumulus DF	3.3	4.3	3.7	3.7	3.3	3.9
Orocide	4.7	3.5	2.8	3.4	3.8	4
Serenade ASO	3.3	3	3.9	2.8	2.8	3.5
Romeo	3.2	3.3	4	3.6	4.3	3.8

Foliage assessment on 0-7 scale, glasshouse test 28/01/21

(L1 and L2 are leaves on the same plant)

Rep 1	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2
UT	7	4	7	6	7	1	5	1	7	5	3	7	2	7	2	6	3	7	1	7	7	6	3	4	7	5	7	5	3	
AHDB9885 Low	1	2	1	0	4	1	1	5	1	2	3	4	2	1	1	2	1	0	1	2	5	7	1	1	1	3	1	4		
AHDB9885 High	1	1	0	0	0	0	0	0	1	2	1	0	1	1	1	1	1	0	1	1	1	0	3	0	6	2	2	0	1	0
Botector	6	1	0	0	7	1	6	1	7	0	2	1	7	1	2	0	6	1	6	1	5	1	0	2	1	0	3	1		
Kumulus	0	2	0	3	5	2	3	2	4	7	3	2	7	2	1	1	7	7	1	2	2	0	2	0	1	1				
Orocide	0	2	1	1	1	1	7	1	3	2	2	0	6	1	5	0	1	0	4	0	7	3	5	1	6	1	4	1	3	1
Serenade	1	1	1	1	5	0	1	2	4	1	5	0	1	1	1	7	0	0	0	0	1	2	1	1	0	7	0	0	0	
Romeo	7	0	7	1	7	3	7	3	7	2	4	1	6	1	6	1	2	2	7	6	7	7	1	5	7	1	1	7	1	
Rep 2																														
UT	7	1	7	1	1	0	1	1	2	1	1	1	3	0	2	1	2	1	2	1	3	1	4	1	1	1	1	1	3	3
AHDB9665 low	1	0	3	1	3	0	1	1	2	0	3	1	5	2	3	0	3	0	7	1	6	1	0	0	1	1	4	3	7	3
AHDB9885 high	2	2	1	0	5	1	7	4	4	2	3	0	7	2	7	1	7	1	7	1	7	0	3	1	4	3	5	1	4	0
Botector	7	1	1	2	7	1	1	2	1	0	4	0	1	2	1	1	0	3	7	1	5	1	2	1	6	1	1	0	0	0
Kumulus	7	1	6	1	2	0	5	0	3	1	2	5	7	0	7	0	7	0	2	1	7	1	1	0	7	0	3	1	1	0
Orocide	7	3	5	1	3	1	7	3	5	2	4	3	7	0	2	1	2	1	4	2	6	1	3	0	2	1	5	1	1	0
Serenade	7	1	2	0	3	1	2	2	0	0	2	1	5	0	2	2	7	1	2	2	7	0	0	0	3	0	3	1	2	1
Romeo	2	0	3	0	2	0	0	0	0	0	3	0	1	0	1	0	3	0	0	0	3	1	1	1	1	0	2	1	3	1
Rep 3																														
UT	5	1	7	1	3	1	3	1	3	2	2	1	4	1	1	1	7	1	1	0	3	1	1	0	4	1				
AHDB9885 low	1	0	1	0	1	1	1	0	1	1	0	0	3	1	2	1	4	0	0	0	3	0	2	1	6	0	1	0	2	1
AHDB9885 high	3	1	4	2	0	0	0	0	7	0	2	0	0	0	5	1	2	1	2	0	2	1	2	0						
Botector	6	0	1	0	1	0	0	0	1	0	1	1	0	0	0	0	0	0	7	0	0	0	3	1	2	0	2	1	2	1
Kumulus	6	1	2	0	5	2	7	3	1	0	1	0	0	0	1	0	7	1	6	0	0	0	3	1	7	0	5	0	2	0
Orocide	3	1	2	1	6	1	1	0	3	1	1	2	4	2	2	1	5	1	5	1										
Serenade	3	0	2	1	1	0	0	0	7	0	2	0	0	0	0	0	4	4	5	0	7	0	3	1	0	0	2	1	1	1
Romeo	3	2	7	1	5	0	3	0	3	0	6	0	2	0	0	0	0	0	3	0	5	0	2	0	3	1	1	2	2	0
Rep 4																														
UT	2	1	4	2	5	3	4	2	1	0	1	1	3	2	7	2	0	0	5	1	7	2	7	1	3	3				
AHDB9885low	2	5	3	3	3	5	4	2	1	0	3	2	5	2	7	2	0	0	0	0	0	0	0	0						
AHDB9885 high	2	1	0	0	0	0	2	0	2	3	0	0	1	0	2	1	2	1	6	1	3	1								
Botector	3	0	1	0	1	0	2	1	2	1	0	0	7	1	3	0	0	0	0	0	6	1	0	0	7	2	6	1	2	1
Kumulus	1	1	1	0	0	0	1	0	4	1	0	0	7	1	0	1	4	0	3	0	5	1	2	0	0	0	0	0	0	0
Orocide	7	2	0	0	6	1	7	2	1	0	2	1	2	1	1	0	7	2	3	2	7	2	7	1						
Serenade	2	1	5	3	5	1	1	0	0	0	7	1	0	0	2	0	2	0	0	0	2	1	3	0						
Romeo	0	0	0	0	3	0	3	0	0	0	2	0	1	0	3	1	0	0	7	0	4	1	2	0	2	1	0	0		
Rep 5																														
UT	1	1	0	0	7	0	2	1	0	0	3	0	1	0	1	0	0	0	3	1	4	1	7	1	6	2	3	3	3	3
AHDB9885 low	0	0	1	6	6	1	0	0	2	2	7	1	6	2	2	0	2	0	4	0	3	1	3	0	3	2	0	0	2	2
AHDB9885 high	6	0	7	5	7	1	7	0	3	0	3	0	7	0	4	0	7	1	0	0	7	3	7	1						
Botector	0	0	0	1	0	0	0	0	0	0	0	0	4	0	3	0	0	0	2	3	6	1	3	2						
Kumulus	7	4	7	0	7	0	7	0	0	0	4	3	5	2	7	0	6	0												
Orocide	7	1	7	1	2	0	1	0	4	0	6	0	5	1	4	0	2	0	1	0	7	0	0	0	1	1	3	1	0	0
Serenade	7	1	3	0	0	0	2	0	7	2	3	1	0	0	0	0	0	3	0	0	0	2	0	0	0	4	0	0		
Romeo	7	0	0	0	3	0	1	0	4	1	2	1	3	1	2	0	2	0	5	0	7	7	6	0						
Rep 6																														
UT	7	1	6	2	5	1	3	1	7	2	7	3	1	0	2	0	1	1	7	0	7	2	6	3	7	4				
AHDB9885low	0	0	0	0	2	1	4	2	3	2	4	1	3	5	0	0	3	2	7	2	7	1	7	7	1	1	7	1	0	0
AHDB9885high	6	3	7	2	7	0	1	1	7	0	7	0	1	2	7	0	0	0	0	0	3	1	0	0						
Botector	5	1	1	1	1	0	1	1	1	1	3	2	2	2	2	1	2	1	6	1	5	2	2	1	2	1	3	2	7	4
Kumulus	0	2	0	0	1	0	7	1	2	0	0	0	0	6	5	0	2	0	0	0	2	0	3	1						
Orocide	2	1	5	1	6	5	6	1	1	1	7	3	7	2	1	2	6	0	7	1	3	0	7	1	2	6	0	5	1	0
Serenade	3	0	0	0	0	0	0	0	3	0	1	0	0	0	2	0	2	1	0	0	0	4	0	5	1	7	1	0		
Romeo	7	0	7	3	0	0	6	0	0	0	0	0	1	0	0	0	4	4	1	3	0	7	4	6	2	1	1	6	3	1

Foliage assessment % leaf area infected, glasshouse test 18/02/21

(L1 and L2 are leaves on the same plant)

Rep 1	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2
UT	33	45	5	37	45	47	55	57	35	37	57	59	75	77	45	57	35	37	39	41	55	57	75	77	83	50	55	53
AHDB9885 low	13	17	23	25	35	33	5	7	11	13	25	23	33	35	45	47	7	35	5	23	25	9	11	13	15	11		
AHDB9885 high	5	7	13	7	15	17	25	33	3	5	11	13	35	55	3	7	15	17	7	9	11	13	5	3	7	9	5	7
Botector	11	9	15	17	23	25	35	37	13	11	5	3	7	11	21	25	30	33	21	17	15	13	17	11	5	21		
Kumulus	33	45	21	57	5	75	25	33	35	45	11	17	5	23	3	37	5	5	13	15	33	35	45	57	35	50		
Orocide	75	35	45	50	11	13	23	25	5	27	3	35	50	55	7	11	33	13	45	11	45	43	50	47	7	11	5	21
Serenade	11	13	5	3	25	35	75	7	35	33	21	23	13	15	5	9	33	35	11	17	15	13	15	13	17	21		
Romeo	17	21	29	25	33	35	3	5	27	55	3	45	5	7	3	5	3	7	11	15	17	17	21	23	9	11		
Rep2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2
UT	60	75	75	80	55	50	33	35	45	75	83	77	79	55	57	53	69	73	75	67	57	77	83	55	75	77	60	55
AHDB9885 low	17	18	25	27	23	25	21	19	28	35	35	37	45	55	15	7	5	3	25	25	17	17	15	9	7	13	15	25
AHDB9885 high	33	35	40	45	37	40	45	43	23	17	55	55	23	17	9	5	5	45	30	33	33	40	45	45	50	47	47	35
Botector	17	5	3	3	23	25	27	35	33	45	65	33	17	55	65	65	33	45	45	55	33	25	17	33	37	40	37	45
Kumulus	65	75	60	45	45	35	33	37	17	23	65	75	75	55	45	67	75	73	70	33	35	17	33	35	35	37	55	65
Orocide	65	75	75	33	7	5	55	50	45	75	65	55	55	77	23	33	75	75	70	65	55	50	70	75	50	55	60	50
Serenade	3	3	5	7	17	23	25	33	45	7	19	23	25	30	21	7	5	45	60	55	17	15	15	11	5	7		
Romeo	21	15	13	11	33	25	7	5	25	33	45	47	50	55	23	21	17	15	33	30	45	37	30	23	25	30	21	17
Rep 3																												
UT	75	77	83	85	55	45	37	65	67	77	75	77	75	83	85	87	75	77	87	83	85	77	75	73				
AHDB9885 low	55	65	77	83	50	55	77	83	75	35	33	23	21	55	65	60	45	43	40	50	55	65	75	53	55	70	75	55
AHDB9885 high	33	23	5	7	13	17	33	35	37	45	45	55	50	37	50	75	50	37	33	35	23							
Botector	45	55	23	30	3	5	35	37	45	50	65	57	20	25	33	37	35	39	45	55	39	35	40	45	33	23	25	33
Kumulus	13	15	30	45	37	39	55	77	85	65	60	57	53	55	65	77	73	85	55	50	37	33	75	77	75	50	45	47
Orocide	77	83	75	77	65	60	55	50	47	33	67	65	13	7	85	87	83	85	77	75	55							
Serenade	11	7	15	17	25	27	35	45	55	65	11	7	13	15	45	55	65	55	23	17	13	25	23	27	35	45	47	30
Romeo	57	65	75	77	33	35	23	27	65	67	75	77	83	85	65	67	55	45	77	75	65	67	53	55	35	37	45	55
Rep 4																												
UT	75	77	65	60	55	57	79	77	85	83	85	55	53	65	60	55	63	65	55	50	55	57	65	77	75	83		
AHDB9885 low	57	35	23	45	75	77	55	53	57	55	50	77	75	79	35	45	50	55	33	25	65	55						
AHDB9885 high	23	17	15	17	35	37	45	55	75	65	35	37	25	23	35	45	30	33										
Botector	11	7	3	5	33	35	33	45	50	55	11	13	23	25	35	37	45	25	11	17	23	25	5	7	23	27	25	30
Kumulus	33	35	37	35	33	27	25	13	15	11	17	35	33	37	55	75	77	75	83	85	75	77	77	85	65	75	77	83
Orocide	65	77	75	85	83	87	75	77	55	50	45	35	33	30	23	27	15	7	13	11								
Serenade	45	35	33	35	45	50	33	75	77	33	17	15	37	35	75	65	55	50	47	43								
Romeo	15	13	75	77	53	55	27	25	75	77	65	55	55	57	45	35	33	37	65	70	55	45	33	37				
Rep 5																												
UT	77	75	83	85	65	63	55	57	55	57	77	83	65	63	87	89	53	35	73	75	33	30	85	81	87	93	53	55
AHDB9885 low	23	25	35	45	50	55	65	60	83	85	23	17	33	35	37	45	55	50	21	17	35	37	50	53	55	57	23	27
AHDB9885 high	25	37	33	35	45	57	65	77	7	5	15	21	37	35	39	43	47	55	50	23	27							
Botector	5	3	7	11	23	25	33	35	21	17	15	33	31	5	7	3	5	11	15	5	7							
Kumulus	75	77	85	83	50	55	35	37	75	77	75	75	73	85	77	75												
Orocide	83	85	77	75	87	85	89	55	75	77	50	55	35	37	77	83	85	87	77	75	73	75	77	65	60	75	77	75
Serenade	33	35	21	17	65	75	55	77	83	85	77	75	50	53	50	47	45	37	39	43	45	50	55	75	55	53		
Romeo	33	31	27	25	55	57	75	73	45	43	33	25	50	55	65	75	35	37	45	43	55	53						
Rep 6																												
UT	85	87	75	77	79	73	75	77	87	93	85	75	65	67	55	50	53	57	47	43	77	75	87	89	91	85	75	77
AHDB9885 low	25	33	45	53	57	65	67	75	17	15	35	33	67	63	57	59	65	67	75	71	75	65	60	55	45	37	43	53
AHDB9885 high	35	23	45	33	50	55	17	15	7	11	35	37	55	65	75	77	23	21	17	25	5	7	23	35				
Botector	37	35	21	27	55	45	57	53	7	11	45	35	77	75	55	65	53	45	57	55	34	37	39	43	47	45	53	47
Kumulus	77	75	55	53	35	37	33	47	77	30	67	60	83	87	91	85	77	73	67	65								
Orocide	43	45	55	57	23	25	77	79	85	87	89	93	45	57	75	77	83	87	85	87	91	87	77	75	85	87	75	89
Serenade	33	35	45	43	23	25	30	35	17	13	5	11	55	53	75	67	57	55	37	25	65	77	75	21	25	11		
Romeo	33	35	37	57	45	34	35	30	75	65	83	87	67	55	45	49	53	53	63	65	71	67	55	50	53	55	57	50

Appendix G ORETO certificate

Certificate of

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

This certifies that

NIAB

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
Stored Crops

Date of issue: **19 March 2018**
Effective date: **29 January 2018**
Expiry date: **28 January 2023**

Signature *Authorised signatory*

Certification Number
ORETO 397

HSE
Chemicals Regulation Division

Department of
Agriculture and
Rural Development