CP 205 AHDB Horticulture Efficacy Trials 2022
Final Trial Report

Work package:	WP 15
Title:	SCEPTREplus - Control of bacterial leaf spots of laurel
Crop	Cherry Laurel
Target	Bacterial leaf spot; *Pseudomonas syringae* pv. *syringae*
Lead researcher:	Erika Wedgwood
Organisation:	ADAS
Period:	18/10/2022 – 13/12/2022
Report date:	27/01/2023
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ORETO Number:	23-06

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 27 January 2023

Author's signature E. F. Wedgwood

Trial Summary

Introduction

The range of plant protections products currently available for the control of bacterial pathogens in hardy nursery stock is very limited. Other control measures include cultural and hygiene strategies, but these practices have limited success.

Prunus laurocerasus, cherry laurel, is a hardy nursery stock species that is susceptible to bacterial shot-hole caused by *Pseudomonas syringae* pv. *syringae.* The damage caused by the pathogen reduces the quality and marketability of plants.
Leafspot and shot-hole together with cankers can also be caused by *Xanthomonas arboricola* pv. *pruni*, but this is an EU quarantine pathogen.

The objective of these trials was to identify crop-safe and efficient plant protection products for use against bacterial shot-hole, with the aim of expanding the range of options available to growers.

Methods

The trial was sited in a glasshouse at a commercial nursery with a history of bacterial leafspots and set up on 18 October 2022 with *P. laurocerasus* (laurel) liners from that nursery in 105 mm diameter pots, with 15 plants per plot. There were nine treatments, including untreated plants which received a water spray. Two commercial standards were used: Serenade ASO (*Bacillus amyloliquefaciens* strain QST 713) Amylo-X (*Bacillus amyloliquefaciens* strain D747). The experimental products comprised two chemical products (AHDB 9711 & AHDB 9710), two microbial bioprotectants (AHDB 9788 & AHDB 9758) and two plant extract bioprotectants (AHDB 9957 & AHDB 9852). The product application timings differed between products based on information provided on their actual or likely label directions (**Table 1**) as did the dose rates. Products were applied overhead using a single 02F110 nozzle by air-assisted knapsack sprayer at 400L/ha, with six application timings at weekly intervals. Pots of laurel plants with bacterial leaf spot were placed within the trial the day after the second application of products, to act as disease spreaders, and the trial overhead irrigated.

Table 1. Treatment application timings and the products applied to laurel, commencing on 18 October, and completing on 22 November 2022.

Treat-ment	Timing 1 (Day 0)	Timing 2 (Day 7)	Timing 3 (Day 14)	Timing 4 (Day 21)	Timing 5 (Day 28)	Timing 6 (Day 35)
1	Untreated	Untreated	Untreated	Untreated	Untreated	Untreated
2	Serenade ASO	Serenade ASO	Serenade ASO	Serenade ASO	Serenade ASO	Serenade ASO
3	AHDB 9711	AHDB 9711	AHDB 9711	AHDB 9711	AHDB 9711	AHDB 9711
4	AHDB 9710	AHDB 9710	AHDB 9710	AHDB 9710	AHDB 9710	AHDB 9710
5	AHDB 9788	AHDB 9788	-	-	-	-
6	AHDB 9758	AHDB 9758	AHDB 9758	AHDB 9758	-	-
7	AHDB 9957	AHDB 9957	AHDB 9957	AHDB 9957	AHDB 9957	AHDB 9957

8	Amylo X	Amylo X	Amylo X	Amylo X	Amylo X	Amylo X
9	AHDB 9730	AHDB 9730	AHDB 9730	AHDB 9730	AHDB 9730	AHDB 9730

The plots were assessed immediately preceding the sprays on 18 October, and then similarly on subsequent application days of 25 October, 1, 8, 15 and 22 November. The final assessment was on 13 December, three weeks after the last applications.

At each of the seven assessments, the leaf area covered by bacterial leaf spot lesions and/or shot-hole was assessed on each of four mature leaves selected at random on each of the 15 plants in a plot. This was supplemented by whole plot assessments of the % of leaf area covered by both leaf spots, together with a plant vigour index and a record of any phytotoxicity.

Results

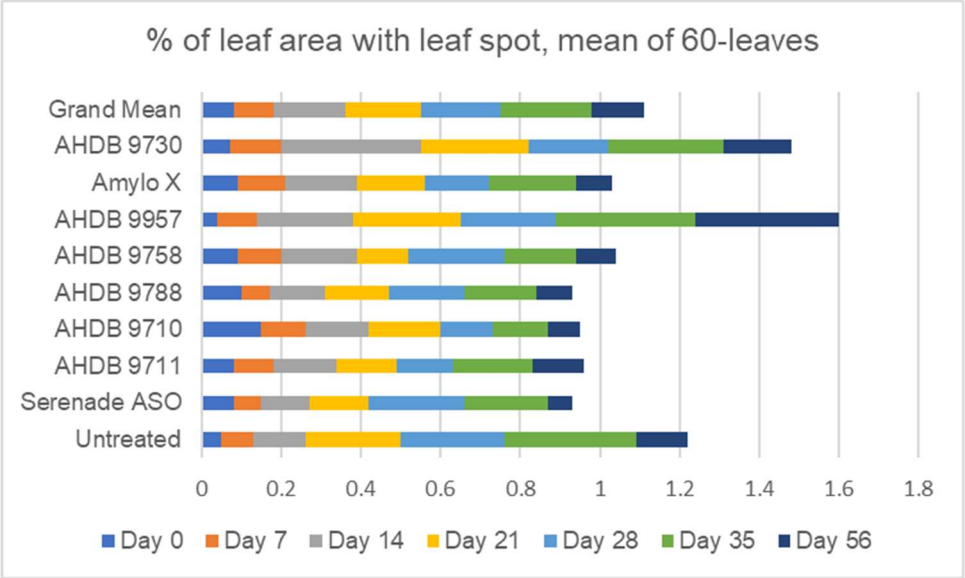

Figure 1. Comparisons of mean % leaf area with leaf-spotting at each of the seven assessment dates based on 60-leaf/plot samples, as shown by each bar segment length scaled on the bottom axis.

With one exception (AHDB 9957 with mean high leaf spot on Day 56), there were no significant differences at any of the assessments between the untreated and any of the standard or experimental products in the mean proportion of leaf area affected by bacterial leafspot based on results from 60 leaves per plot. Levels following the pre-treatment assessment (Day 0) were mainly below a mean of 0.2% per leaf (as shown by the bar lengths in **Figure 1**).

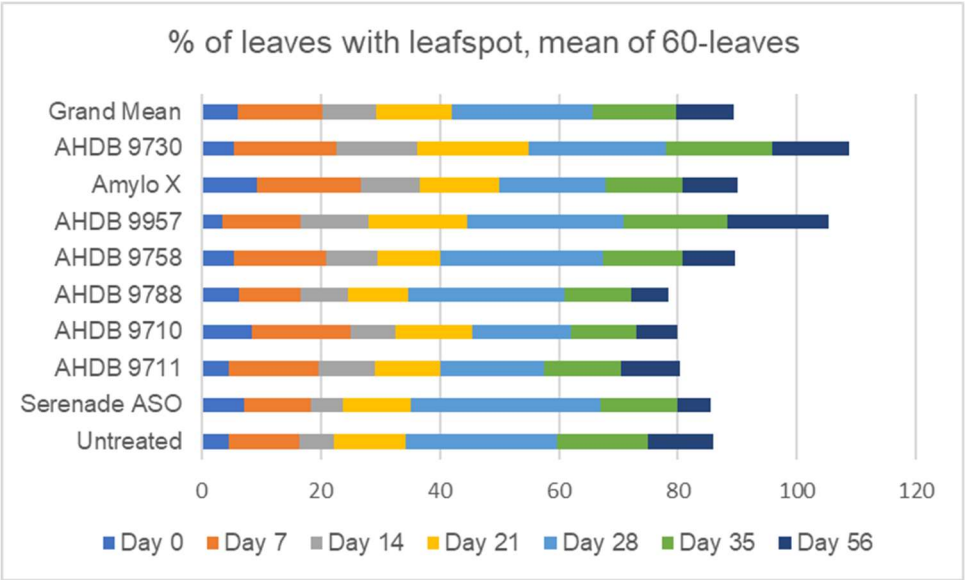

Figure 2. Comparisons of mean % of leaves with leaf-spotting at each of the seven assessment dates based on 60-leaf/plot samples, as shown by each bar segment length scaled on the bottom axis.

With one exception (AHDB 9957 with mean high leaf spot on Day 56), there were no significant differences at any of the assessments between the untreated and any of the standard or experimental products in the mean proportion of leaves affected by bacterial two leafspot based on results from 60 leaves per plot. Initially, overall, a mean 6% of leaves had spotting or shot-hole, rising to 23% by Day 28. By the final assessment on Day 56, 8% of leaves were affected, however the leaves scored were not necessarily the same at each assessment (**Figure 2**).

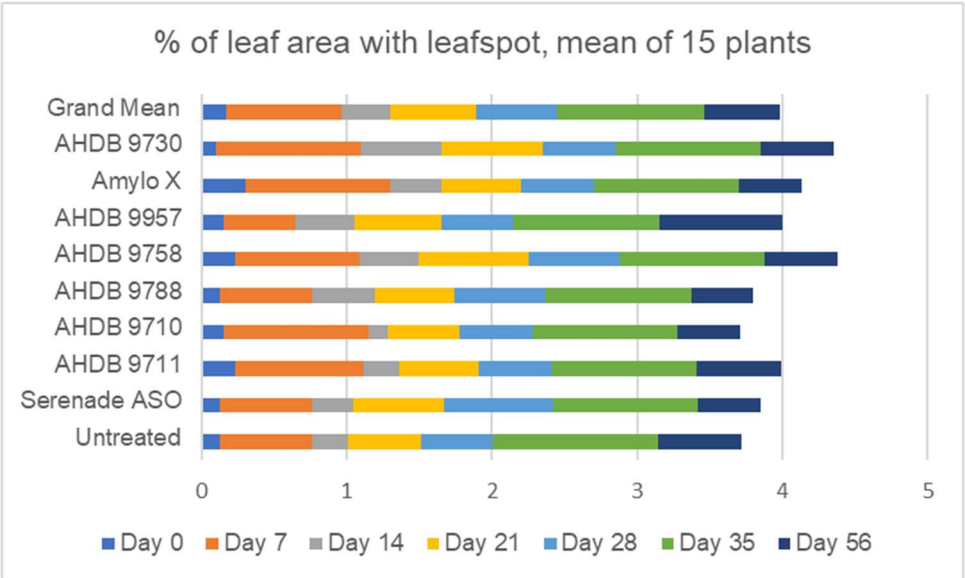

Figure 3. Comparisons of mean % leaf area with leaf-spotting at each of the seven assessment dates based on 15 plants / plot samples, as shown by each bar segment length scaled on the bottom axis.

For the whole-plot assessments, there were no significant differences between leaf spot coverage in the untreated, standard product and experimental product treatments at any of the seven assessments. Assessment across whole plots (**Figure 3**), gave

disease severity similar to that found from the 60-leaf samples (**Figure 1**) i.e., mainly below 0.8%, except on Day 35 when 1% of leaves were affected across plots.

None of the products caused any phytotoxicity to the laurel plants. However, product AHDB 9710 left a white deposit that increased in thickness over the trial period applications even though plants received overhead irrigation.

Take home message:
None of the experimental treatments can be recommended for use against bacterial leaf spot on laurel as they did not offer any significant reduction in disease compared with the untreated. There were, however, also no differences in leaf spotting incidence or severity between the experimental products and the standard conventional products Amylo-X and Serenade ASO. There was little change in symptom severity or incidence over the eight weeks and so there may have been little new infection during the period of the trial to evaluate the control by protectant products.

SCIENCE SECTION

Objectives
To screen plant protection products (chemical, microbial and botanical), for efficacy against bacterial leaf spot of laurel and any phytotoxicity.

Methods

The trial was sited at a commercial nursery and set up in a glasshouse on 18 October 2022 with *Prunus laurocerasus* liners from that nursery in 10.5 cm diameter pots. Each plot comprised a plastic carry-tray with drainage holes with three lines of five pots of plants, with about 20 mm space between plants (**Tables 3 & 4**). The layout and a photograph of the trial are given in the Appendix (**Figure 1 & 2**). A randomised block design was used for the trial layout, with nine treatments including an untreated control in each of four replicate columns of trays (**Table 5**).

Trial plants were infected using spreader plants (laurel plants that were naturally infected on the same site), which were introduced on the 8th day after the first spray application and were spread evenly throughout the trial, with one spreader pot in the 300 mm space between each plot within each replicate and at the ends (Appendix **Figure 3**). The spreader plants were kept free from any experimental product applications and remained in-situ until after the final treatment assessments. Following standard nursery practice, the trial received overhead irrigation according to the needs of the plants. A “rain” gauge was placed within the crop to monitor water application.

Prior to the trial, the plants had all received prophylactic treatment by the nursery with a sequence of single applications at seven-day intervals of Previcur Energy (propamocarb) against downy mildew, DiPel DF (*Bacillus thuringiensis* subs. *kurstaki*) against lepidopterous larvae, Amylo-X WG (*Bacillus amyloliquefaciens* subsp. *plantarum* strain D747), and Serenade ASO (*Bacillus amyloliquefaciens* strain QST 713) against fungal diseases. The last nursery application was made on 14 September 2022, a month before experimental product applications commenced.

All eight of the treatments were protectant products and so first applied at the start of the trial at Day 0. Due to potential label restrictions AHDB 9788 was restricted to the

two applications before introduction of the spreader plants, but the other five experimental and the standard products Serenade ASO and Amylo-X were all applied six times (**Table 1 & 6**). Dose rates were as on the product label or, for the experimental products, as agreed with the product suppliers (**Table 6**). All treatments were applied at 7-day intervals using a single 02F110 nozzle and an air-assisted knapsack sprayed at 400 L/ha water volume and were sprayed directly above each plant, without run-off (**Table 7**). Due to the large leaves on laurel, which shield those below, good coverage of the lower leaves was not possible. The applied chemicals were mixed directly before spraying (at most 20 minutes before).

The plots were assessed on seven different occasions (**Table 8**) for disease incidence and severity, vigour and phytotoxicity. The assessments were carried out at the time of application of the first treatment, and again at 7-day intervals prior to each treatment application (six applications) and then after three weeks i.e., at 56 days after the first products were applied in the trial.

Two disease assessments were performed: 1) 60-leaf assessments in which four leaves per plant on each of the 15 plants in a plot were assessed and individually recorded for the % cover of bacterial leaf spotting or holes left by the lesion centres desiccating and falling out (shot-holing). This assessment thus also recorded the proportion of leaves affected per plot, and 2) whole plot assessments in which the % of leaf area covered by leaf spots or shot-holing was assessed across all the pots in a plot. Photographs giving examples of the % cover of each of the leaf spots are given in Appendix **Figures 4 & 5**.

Whole plot vigour was assessed using a 0 (dead) to 9 (excellent) index which considered factors including plant canopy density, the production of new growth and leaf colouration. The plants were examined for any phytotoxicity, for example distortion or stunting. Records were made of any spray deposit residues remaining on the plants.

Trial conduct

Table 2: EPPO guidelines followed. As no guideline was available for bacterial leaf spot on hardy nursery stock the closest guidelines were examined, and a variation as detailed in the table made.

Relevant EPPO guideline(s)		**Variation from EPPO**
EPPO PP1/135 (4)	Phytotoxicity assessment	No variation
EPPO PP1/152 (4)	Guideline on design and analysis of efficacy evaluation trials	No variation
EPPO PP1/225 (2)	Minimum effective dose	No variation
EPPO PP1/181 (4)	Conduct and reporting of efficacy evaluation trials including good experimental practice	No variation
EPPO PP 1/214(3)	Principles of acceptable efficacy	No variation
EPPO PP 1/224(2)	Principles of efficacy evaluation for minor uses	No variation

EPPO PP 1/196(2)	Efficacy evaluation of fungicides Fungi on woody ornamentals	Fungi on woody ornamentals: From each plot select at random at least 50 leaves of similar age. Record the level of infection: number of infected leaves and percentage of leaf area affected. A scale may be used and, if so, should be described. Variation: 4 mature leaves at random from each of 15 plants/plot given a % leaf spot / necrosis / shot-hole area score: % of leaves affected in the sample calculated. Whole plot assessment of % area of any leaf spotting / necrosis / shot-holing
EPPO PP 1/221(1)	Efficacy evaluation of fungicides. Foliar diseases on non-woody ornamentals	Foliar diseases on non-woody ornamentals: At least 10 plants or shoots should be selected in each plot. If infection on plants is uneven, at least 5 leaves per plant or shoot should be selected at the position where infection occurs on the untreated plants. The level of infection should be recorded as number of infected leaves and percentage of leaf area infected. A scale may be used and, if so, should be described. If infection on plants is even, it is also possible to assess infection of the whole plant. If plants with very small leaves are used, the level of infection should be estimated for whole shoots. Variation: as given above.
EPPO PP 1/318 (1)	Efficacy evaluation of fungicides. *Pseudomonas syringae* pv. *syringae* and pv. *morsprunorum* on stone fruit.	Assessment on leaves: For each plot, the percentage of infected leaves and percentage of leaf area affected should be assessed on at least 100 leaves randomly selected. Variation: as given above.

Test site

Table 3: Description of trial location and husbandry

Item	Details
Location address	Wyevale Hereford Kings Acre Road, Hereford, Herefordshire HR4 0SE. Grid Reference SO 47300 42118.
Crop	Cherry Laurel
Cultivar	*Prunus laurocerasus*
Soil or substrate type	Peat based growing-media. pH 6.1. P 71 mg/L (index 5), K 235 mg/L (index 2) Mg 486 mg/L (index 6). Organic matter 87.3%. on 14/11/2022
Agronomic practice	Standard glasshouse-grown container crop with overhead (fixed and hosepipe) irrigation. Frost-protection using air blast heating.
Prior history of site	The target diseases have been reported from the nursery.

Trial design

Table 4: Trial layout and number of plants

Item	Details
Trial design:	Randomised block
Number of replicates:	4
Row spacing:	0.3 m between plots & 0.4m between blocks
Plot size: (w x l)	0.31m x 0.56m (pots stood in a 15-hole carry-tray)
Plot size: (m^2)	0.17
Number of plants per plot:	15 in 10.5 cm diameter pots

The plot layout (**Figure 1**) and photographs of the plots are given in the Appendix (**Figures 1, 2, 3 & 4**).

Treatment details

Table 5: Products, with experimental products coded, together with the quantity of active substance in the product and the product formulation type. Active ingredients are only given for authorised products, otherwise the nature or the substance is given.

AHDB Code	Active substance	Product name/ manufacturer code	Formulation batch number	Content of active substance in product	Formulation type	Adjuvant
No need	*Bacillus amyloliquefaciens* (former *subtilis*) strain QST 713	Serenade ASO (MAPP 16139)	EZU2015707	1015.1 g/L	EC	N
AHDB 9711	Confidential					N
AHDB 9710	Confidential					N
AHDB 9788	Confidential					N
AHDB 9758	Confidential					N, but a buffer
AHDB 9957	Confidential					N
No need	*Bacillus amyloliquefaciens* strain D747	Amylo-X WG (MAPP 17978)	2591462	$5x10^{13}$ CFU /kg	WDG	N
AHDB 9730	Confidential					N

Application schedule

Table 6: Treatments, the application rates used. The application slots each product had in the treatment are shown (the application dates are given in **Table 7**).

Treatment number	Treatment: product name or AHDB code	Rate of product (l or kg/ha)	Application code
1	Untreated (water sprayed)	n.a.	A,B,C,D,E,F
2	Serenade ASO	10 L	A,B,C,D,E,F
3	AHDB 9711	6 L (1.5%)	A,B,C,D,E,F
4	AHDB 9710	4 L	A,B,C,D,E,F
5	AHDB 9788	0.2 kg	A,B
6	AHDB 9758 + buffer	0.75 kg + 3 kg	A,B,C,D
7	AHDB 9957	3 L	A,B,C,D,E,F
8	Amylo X	2.5 kg	A,B,C,D,E,F
9	AHDB 9730	1.6 L (0.4%)	A,B,C,D,E,F

Application details

Table 7: Crop growth, spray equipment and environmental conditions in the glasshouse for each of the weekly application dates.

	Application A	Application B	Application C	Application D	Application E	Application F
Application date	18/10/22	25/10/22	1/11/22	8/11/22	15/11/22	22/11/22
Time of day	13:20	12:15	12:00	12:40	11:44	13:15
Crop growth stage (Max, min average BBCH)	Branching	Branching	Branching	Branching	Branching	Branching
Crop height (cm)	10-25	20-40	25-40	20-30	25-40	30
Crop coverage (%)*	100%	100%	100%	100%	100%	100%
Application Method	Foliar	Foliar	Foliar	Foliar	Foliar	Foliar
Application Placement	Spray	Spray	Spray	Spray	Spray	Spray
Application equipment	Oxford sprayer	Oxford sprayer	Oxford sprayer	Oxford sprayer	Oxford sprayer	Oxford sprayer
Nozzle pressure (bar)	3	3	3	3	3	3
Nozzle type	Hypro	Hypro	Hypro	Hypro	Hypro	Hypro
Nozzle size	02F110	02F110	02F110	02F110	02F110	02F110
Application water volume/ha	400	400	400	400	400	400
Temperature of air - shade (°C)	19.9	18.55	13.15	12.85	11.8	9.75
Relative humidity (%)	63.8	79.35	91.38	83.05	89	83.1
Wind speed range (m/s)	n/a	n/a	n/a	n/a	n/a	n/a
Dew presence (Y/N)	N	N	N	N	N	N
Wetness of soil - 2-5 cm	Damp	Damp	Damp	Damp	Damp	Damp
Cloud cover (%)	0	75	75	100	100	0

* Foliage ground cover was mostly 100% per plant pot with some leaves overlapping between pots from the start to give 100% ground cover in the plot.

Assessment details

Table 8: Evaluation timings following the first of two applications of the two conventional plant protection products on 1 November. All the experimental products were microbial or chemical biopesticides with all but one product (AHDB 9714) first being applied on 18 October (as detailed in **Table 1** &**Table 7**).

	Evaluation Timing (DA)*				
Evaluation date	**After first conventional insecticides**	**After first bio-pesticides**	**Crop Growth Stage (BBCH)**	**Evaluation type (efficacy, phytotoxicity)**	**Assessment**
18/10/2022 (Day 0)	0	0	branching	Efficacy	% of leaf area with leaf spot, necrosis or shot-hole per leaf from a sample of 60 leaves/plot. % of leaf area covered by leaf spot, necrosis or shot-hole per plot
				Vigour	0 to 9 (dead to excellent) indices
				Phytotoxicity	% of leaf area affected
25/10/2022 (Day7)	7	7	branching	Efficacy	% of leaf area with leaf spot, necrosis or shot-hole per leaf from a sample of 60 leaves/plot. % of leaf area covered by leaf spot, necrosis or shot-hole per plot
				Vigour	0 to 9 (dead to excellent) indices
				Phytotoxicity	% of leaf area affected
01/11/2022 (Day14)	14	14	branching	Efficacy	% of leaf area with leaf spot, necrosis or shot-hole per leaf from a sample of 60 leaves/plot. % of leaf area covered by leaf spot, necrosis or shot-hole per plot
				Vigour	0 to 9 (dead to excellent) indices
				Phytotoxicity	% of leaf area affected
08/11/2022 (Day 21)	21	21	branching	Efficacy	% of leaf area with leaf spot, necrosis or shot-hole per leaf from a sample of 60 leaves/plot. % of leaf area covered by leaf spot, necrosis or shot-hole per plot
				Vigour	0 to 9 (dead to excellent) indices
				Phytotoxicity	% of leaf area affected
15/11/2022 (Day 28)	28	28	branching	Efficacy	% of leaf area with leaf spot, necrosis or shot-hole per leaf from a sample of 60 leaves/plot. % of leaf area covered by leaf spot, necrosis or shot-hole per plot
				Vigour	0 to 9 (dead to excellent) indices
				Phytotoxicity	% of leaf area affected
22/11/2022 (Day 35)	35	35	branching	Efficacy	% of leaf area with leaf spot, necrosis or shot-hole per leaf from a sample of 60

					leaves/plot. % of leaf area covered by leaf spot, necrosis or shot-hole per plot
				Vigour	0 to 9 (dead to excellent) indices
				Phytotoxicity	% of leaf area affected
13/12/2022 (Day 56)	42	42	branching	Efficacy	% of leaf area with leaf spot, necrosis or shot-hole per leaf from a sample of 60 leaves/plot. % of leaf area covered by leaf spot, necrosis or shot-hole per plot
				Vigour	0 to 9 (dead to excellent) indices
				Phytotoxicity	% of leaf area affected

* DA – days after application

The whole-plot assessments can differ from separate leaf assessments as human bias when sampling leaves can result in selection of affected leaves rather than random sampling of mature leaves. Leaves for the 60-leaf assessments were scored on the plant, the leaves were not detached. Results for the same assessment method were valid for comparison between treatments.

Statistical analysis

This experiment used a randomised block design and comprised nine treatments, including an untreated control. There were four replicates for each treatment.

Treatment means and Analysis of Variance were calculated for the disease incidence and severity scores from the four leaves of the 15 plants per plot and the % leaf area covered by leaf spot per plot. In addition, Duncan's multiple range test was used to rank the mean disease levels per treatment.

Vigour scores did not differ between replicates or treatments, and so these were not statistically analysed.

Genstat (21st edition) was used by Chris Dyer to do ANOVA on the data.

Results

Phytotoxicity

No phytotoxic affects were recorded for any of the applied treatments during this trial. However, there was a very obvious white paint-like coating left on the leaves following each application of AHDB 9710 (**Figure 4**).

Vigour

Vigour scores were the same for all the treatments per assessment (**Table 9**). There was a uniform increase in vigour following good growing conditions preceding the mid-November score (with air temperatures at plant height of around 14°C, as shown by logger data in Appendix **Figure 6)**.

Table 9. Mean vigour scores per plot assessed at seven-day intervals from the day of first treatment applications and finally at 21 days after the last treatment was applied.

Mean plant vigour per plot (0-9, where 9=excellent)							
Treatment	**Day 0**	**Day 7**	**Day 14**	**Day 21**	**Day 28**	**Day 35**	**Day 56**
	18/10/22	25/10/22	01/11/22	08/11/22	15/11/22	22/11/22	13/12/22
Untreated	5	5	5	5	7	5	6
Serenade ASO	5	5	5	5	7	5	6
AHDB 9711	5	5	5	5	7	5	6
AHDB 9710	5	5	5	5	7	5	6
AHDB 9788	5	5	5	5	7	5	6
AHDB 9758	5	5	5	5	7	5	6
AHDB 9957	5	5	5	5	7	5	6
Amylo X	5	5	5	5	7	5	6
AHDB 9730	5	5	5	5	7	5	6
Grand Mean	5	5	5	5	7	5	6
F- Probability value	n.sig.	n.sig.	n.sig.	n.sig.	n.sig.	n.sig.	n.sig.
Least significant difference	0	0	0	0	0	0	0
Degrees of freedom	24	24	24	24	24	24	24

n.sig. = scores, and thus mean values, were identical and so ANOVA was not performed.

Disease control

The initial incidence of infection, of 4.6%, for the plots that then remained untreated had doubled by the finish (**Table 10**). However, the laurel leaves were large and so the symptoms only covered around a mean 0.1% of leaf area. **(Table 10)**.

Before the experimental applications commenced, plants in all treatments had a small amount of leaf spotting (**Tables 11 & 12**). At Day 0 across all nine treatments a mean 0.08% of leaf area had leaf spotting, based on individual leaf assessments (**Table 11**) and 0.17% on whole plot estimations (**Table 13**) with a mean 6.0% of leaves affected based on 60-leaf records (**Table 12**). Leaf spotting at the start could not be avoided as the nursery site and laurel species were chosen for their predisposition to leaf spotting and because individual laurel leaves are evergreen there was a likelihood of historical leaf spotting.

Table 10. Untreated levels of pathogens (severity and incidence) at application and by the end of the assessment period using the records for 60 leaves examined per plot.

Common name	**Scientific Name**	**EPPO Code**	**Infestation level pre-application 18/10/2022**	**Infestation level at assessment period start 18/10/2022**	**Infestation level at assessment period end 13/12/2022**
Bacterial Spot	*Pseudomonas syringae* pv. *syringae*	PSDMSY	0.05% leaf area spotted. 4.6% of leaves.	0.05% leaf area spotted. 4.6% of leaves.	0.13% leaf area spotted. 10.8% of leaves.

Following ANOVA statistical analysis of the 60-leaf per plot scores, leaf spot coverage, of 0.36%, for AHDB 9957 was by the last assessment highly significantly (P=0.001) worse than for other treatments including the untreated control. The treatment's preceding four assessments had also shown above average levels (**Table 11**). AHDB 9957 ranked significantly lower than all other treatments using Duncan's test.

The proportion of sampled leaves with any leaf spotting across the treatments rose to a mean 23.6% on Day 28 based on the examination of 60 leaves (**Table 12**). This reduced the marketability of the plants even though the leaf area affected was only 0.2% (**Table 11**). By the final assessment the incidence of leaves with leaf spotting across all treatments was a mean 9.7%, but this was raised by the significantly higher incidence (P =0.014), of 17.0%, in AHDB 9957 (**Table 12**). AHDB 9730 had the next highest, 12.9%, of leaves with leaf spotting and Serenade ASO at 5.42% had significantly fewer leaves affected than this product (**Table 12**) when ranking was compared using Duncan's test.

There were no statistically significant differences in the % leaf area affected when scored across whole plots (**Table 13**). The mean area affected across the treatments rose to no more than 1% from Day 7 of the assessment period **(Table 13).**

Table 11. Mean disease severity per 60 leaves assessed at seven-day intervals from the first treatment applications and finally at 21 days after the last treatment was applied. Mean proportion of leaf area covered by leaf spotting, necrosis or shot-hole, on the leaves assessed.

Mean % leaf area with leaf spotting based on 60 leaves examined per plot							
Treatment	**Day 0** 18/10/22	**Day 7** 25/10/22	**Day 14** 01/11/22	**Day 21** 08/11/22	**Day 28** 15/11/22	**Day 35** 22/11/22	**Day 56** 13/12/22
Untreated	0.05	0.08	0.13	0.24	0.26	0.33	0.13
Serenade ASO	0.08	0.07	0.12	0.15	0.24	0.21	0.06
AHDB 9711	0.08	0.10	0.16	0.15	0.14	0.20	0.13
AHDB 9710	0.15	0.11	0.16	0.18	0.13	0.14	0.08
AHDB 9788	0.10	0.07	0.14	0.16	0.19	0.18	0.09
AHDB 9758	0.09	0.11	0.19	0.13	0.24	0.18	0.10
AHDB 9957	0.04	0.10	0.24	0.27	0.24	0.35	**0.36**
Amylo X	0.09	0.12	0.18	0.17	0.16	0.22	0.09
AHDB 9730	0.07	0.13	0.35	0.27	0.20	0.29	0.17
Grand Mean	0.08	0.10	0.18	0.19	0.20	0.23	0.13
F- Probability value	0.283	0.41	0.509	0.646	0.721	0.216	0.001
Least significant difference	0.083	0.064	0.216	0.178	0.167	0.177	0.119
Degrees of freedom	24	24	24	24	24	24	24

Table 12. Mean disease incidence per 60 leaves assessed at seven-day intervals and finally at 21 days after the last treatment was applied. Mean proportion (%) of the leaves assessed that had leaf spotting, necrosis or shot-hole. Day 0 was before any spray applications.

Mean % of 60 leaves examined per plot which had bacterial leaf spot							
Treatment	**Day 0** 18/10/22	**Day 7** 25/10/22	**Day 14** 01/11/22	**Day 21** 08/11/22	**Day 28** 15/11/22	**Day 35** 22/11/22	**Day 56** 13/12/22
Untreated	4.58	11.67	5.83	12.08	25.42	15.42	10.83
Serenade ASO	7.08	11.25	5.42	11.25	32.08	12.92	5.42
AHDB 9711	4.58	15.00	9.58	10.83	17.50	12.92	10.00
AHDB 9710	8.33	16.67	7.50	12.92	16.67	10.83	7.08
AHDB 9788	6.25	10.42	7.92	10.00	26.25	11.25	6.25

AHDB 9758	5.42	15.42	8.75	10.42	27.50	13.33	8.75
AHDB 9957	3.33	13.33	11.25	16.67	26.25	17.50	**17.08**
Amylo X	9.17	17.50	10.00	13.33	17.92	12.92	9.17
AHDB 9730	5.42	17.08	13.75	18.75	22.92	17.92	12.92
Grand Mean	6.02	14.26	8.89	12.92	23.61	13.89	9.72
F- Probability value	0.460	0.385	0.563	0.403	0.381	0.590	0.014
Least significant difference	5.505	7.398	8.272	8.315	14.499	8.122	5.943
Degrees of freedom	24	24	24	24	24	24	24

Table 13. Whole plot assessments of percentage of leaf area with leaf spotting by either disease estimated to obtain treatment means. Assessed at seven-day intervals from the first treatment applications and finally at 21 days after the last treatment was applied. Day 0 was before any spray applications.

Mean % leaf area with leaf spotting per plot across all leaves							
Treatment	**Day 0** 18/10/22	**Day 7** 25/10/22	**Day 14** 01/11/22	**Day 21** 08/11/22	**Day 28** 15/11/22	**Day 35** 22/11/22	**Day 56** 13/12/22
Untreated	0.13	0.63	0.25	0.50	0.50	1.13	0.58
Serenade ASO	0.13	0.63	0.28	0.63	0.75	1.00	0.43
AHDB 9711	0.23	0.88	0.25	0.55	0.50	1.00	0.58
AHDB 9710	0.15	1.00	0.13	0.50	0.50	1.00	0.43
AHDB 9788	0.13	0.63	0.43	0.55	0.63	1.00	0.43
AHDB 9758	0.23	0.86	0.40	0.76	0.63	1.00	0.50
AHDB 9957	0.15	0.50	0.40	0.60	0.50	1.00	0.85
Amylo X	0.30	1.00	0.35	0.55	0.50	1.00	0.43
AHDB 9730	0.10	1.00	0.55	0.70	0.50	1.00	0.50
Grand Mean	0.169	0.792	0.336	0.592	0.556	1.014	0.522
F- Probability value	0.617	0.722	0.184	0.714	0.656	0.461	0.001
Least significant difference	0.216	0.711	0.291	0.314	0.308	0.122	0.184
Degrees of freedom	24	24	24	24	24	24	24

Discussion

None of the applied treatments offered any statistically significant reduction in disease. Although one treatment, AHDB 9957 had a statistically significantly greater incidence and severity of disease at the last assessment, this was only from the 60-leaf sample, and it is possible that there could have been subconscious selection of the same more-badly affected leaves when recording. This shows that using the two different scoring methods had merit, whereas the detailed examination of leaves can ensure leaf spots are not missed, the plot overview can give a less biased assessment and considers leaves at all positions on the plant.

Although no phytotoxic activity was recorded for any of the treatments, plants treated with AHDB 9852 had a thick paint like residue coating. This was visible after the first spray and got progressively worse with each additional application, as the residue was

not washed off at all by the irrigation between applications. This is concerning as it prevents the plants from being sold. There was no other obvious residue from any of the other products used.
The symptoms that were present at a low level at the start of the experiment would not be expected to be eradicated by any product. Although conditions were suitable for disease development (warm and humid, with water splash for spread from spreader pots and between closely spaced foliage) there was negligible symptom development over the period of the trial even in the untreated. Because laurel leaves are evergreen, any nursery application of a curative fungicide in early 2022 could have reduced pathogen mycelium viability within the visible spots and so even though conditions were conducive for infection there may have been little or no spore production.

Conclusions

Based on the outcomes of this trial, none of the included treatments can be recommended for use against bacterial leaf spot on laurel.

All products except AHDB 9710 were shown to not have any detrimental effects on marketability and so would be acceptable for inclusion in any further trials on this host plant species.

Acknowledgements

ADAS is grateful to the host nursery for being willing to participate in this project and for the supply of plants, providing a glasshouse area and watering. Also, the co-operation of plant protection company representatives for providing information on their products and supplying samples for testing.

Appendix

Trial assessments & sprays conducted by ADAS Rosemaund staff led by Gabriella Parcell.

Date	Trial Diary
17/08/2022	Nursery application of Previcur Energy downy mildew fungicide
31/08/2022	Nursery application of DipPel DF insecticide
07/09/2022	Nursery application of Amylo X protectant fungicide
14/09/2022	Nursery application of Serenade ASO protectant fungicide
18/10/2022	Trial set-up. Spray and assessment 1 complete. Trial will be irrigated every Monday if needed. Trt 4 (AHDB 9710) leaves a paint like residue on the plants
25/10/2022	Spray and assessment 2 complete. 0.5mm of water in the rain gauges. Trt 4 still have obvious residue on the leaves.

26/10/2022	Spreader plants put out in trial
01/11/2022	Spray and assessment 3 complete. Rain gauges: 1.7, 3.7, 5 and 3mm. Spreader plants removed before spray and returned afterwards.
08/11/2022	Spray and assessment 4 completed. No water in rain gauges
15/11/2022	Spray and assessment 5 completed. No water in rain gauges as watered using spot and spray method.
22/11/2022	Spray and assessment 6 complete. No water in rain gauges. Requested change from hosepipe to overhead irrigation using irrigation headers.
13/12/2022	Final assessment done. Data loggers removed and downloaded. 5.5mm and 6.5mm in rain gauges. Three hours reported spent irrigating by the nursery staff over the trial period

Assessments and Application record raw data source on ADAS Boxworth Local Area Network

\\bw\DATA\data\HORTIC\1022248 - RM-23-002 - AHDB Bacterial leaf spot of ornamentals efficacy trial\RM23-002\Data file - SHE planRM23-002 HNS Leaf Spot Bacterial- Datafile 01.xls

Photographs for each assessment date are on the ADAS Rosemaund Local Area Network

\\RM\data\Arable 2023\Horticulture\RM23-002 HNS Leaf Spot Bacterial

TREATMENT	9	5	4	2	8	1	6	3	7
BLOCK	4	4	4	4	4	4	4	4	4
PLOT	401	402	403	404	405	406	407	408	409
TREATMENT	3	7	5	2	4	9	6	1	8
BLOCK	3	3	3	3	3	3	3	3	3
PLOT	301	302	303	304	305	306	307	308	309
TREATMENT	7	1	6	3	4	8	5	9	2
BLOCK	2	2	2	2	2	2	2	2	2
PLOT	201	202	203	204	205	206	207	208	209
TREATMENT	4	9	8	3	5	6	2	1	7
BLOCK	1	1	1	1	1	1	1	1	1
PLOT	101	102	103	104	105	106	107	108	109

Figure 1. Trial design: Layout of laurel plots in the glasshouse, with 15 plants per plot randomised within each of four lines of carry-trays. October to December 2022

Figure 2. Laurel plots in the glasshouse, with 15 plants per plot randomised within each of four lines of nine carry-trays on 18 October 2022. The data logger was held in the terracotta-coloured screen.

Figure 3. Spreader plants positioned between laurel plots. Data logger screen and rain gauge in position. 26 October 2022.

Figure 4. Plot 101 of laurel plants treated with AHDB 9710 on 18 October (left-hand photograph) and with white residue still present on 13 December 2022 (right-hand photograph).

Figure 5. Laurel leaves with bacterial leaf spotting. The left-hand photograph has a leaf with 1% of the leaf affected and 5% of the leaf is affected in right-hand photograph.

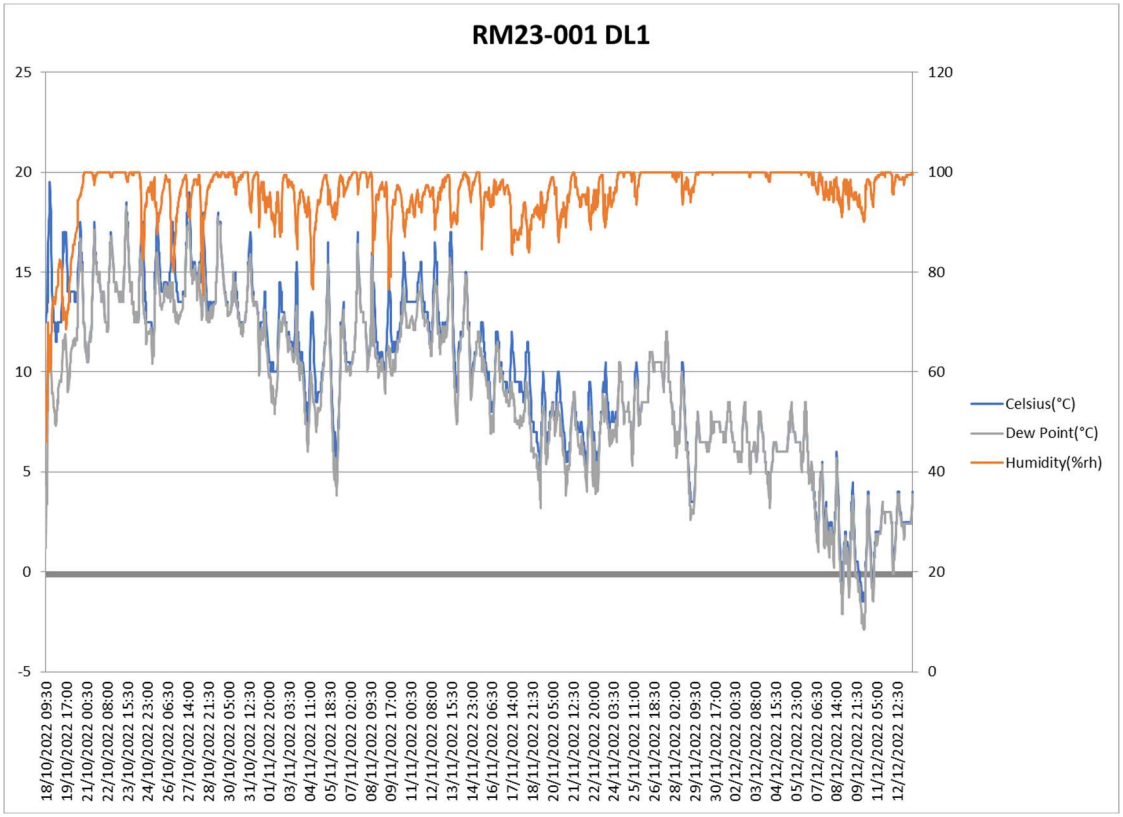

Figure 6. Data logger RM23-002 half-hourly records of air temperature (left-hand axis and blue lines) and humidity (right-hand axis and upper, orange lines) from within a ventilated screen in the nursery glasshouse next to a laurel plot at plant canopy level. Recorded from set up on 18 October 2022 to final assessment on the 13 December. Dew point = calculated times when high humidity and surface temperature would have been conducive to condensation forming on the leaves.

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

This certifies that

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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:

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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
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