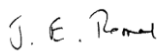
SCEPTREPLUS

Final Trial Report

Trial code:	SP46
Title:	Controlling Pythium and Rhizoctonia in protected baby leaf salad crops
Crop	Baby leaf spinach. The report may also be relevant to protected lettuce, protected herbs, and other baby leaf species such as chard, red beet, lambs lettuce, and rocket
Target	*Pythium ultimum* and *Rhizoctonia solani*
Lead researcher:	Jane Thomas
Organisation:	NIAB
Period:	August 2019 – March 2020
Report date:	September 2020
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

29th September



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Trial Summary

Two small plot trials to test product efficacy against *Pythium ultimum* and *Rhizoctonia solani* on a protected salad leaf crop were established on either side of a soil-bedded glasshouse at NIAB, Cambridge. Inoculum of *P. ultimum* was produced on autoclaved pearl millet, and that of *R. solani* on maizemeal and vermiculite. Both inoculum substrates were carefully raked in to the top 5 cm of soil. A pathway approximately 0.3m wide was kept free of inoculum between the two sides. Untreated seed of a baby leaf spinach variety was drilled mechanically to give a seed rate equivalent to 8 million seeds/hectare 7 days after inoculation. Treatments were applied and re-applied according to discussions with manufacturers and label recommendations. The crop was maintained by overhead watering, and sodium lights were used to supplement natural daylight to give a 14h day. There were four replications of conventional products and six for biological treatments. Plant number, fresh weight, and disease symptoms were assessed at harvest, 38 days after planting. Typical symptoms of *Pythium* infection were recorded, including reduced plant numbers, lower fresh weights per unit area, and pale brown watery rotting of roots. Symptoms of *Rhizoctonia* also manifested as loss in plant number and lower weights, but with distinctive brown markings at the stem base and top of the root and occasionally on the lower leaves. Significant differences ($p<0.05$) were observed between some treatments and the untreated control in each trial. Biological treatments reduced symptom expression in both trials, but the non-biological products that were tested performed better and were as effective or more so than the commercial standard products used.

Introduction

Baby leaf salads have become increasingly popular either in mixes or as single species bagged products. Year round production can be achieved with protected cropping, but intensive use of soils leads to the build up of soil-borne pathogens. In particular, *Pythium ultimum* (damping off and root rot) and *Rhizoctonia solani* (root rot) have caused problems for growers. While products have been investigated for their control in outdoor cropping (eg for *Pythium* in AHDB project FV367), there has been no equivalent evaluation in protected cropping.

Soil-borne pathogens are inherently heterogeneous in distribution, and usually occur as a complex. In a single site and season on naturally infected soils, it may be impossible to obtain informative results suitable for future product registration. Inoculation with pathogens increased on artificial media and applied to a relatively small area can create a much more uniform test facility with sufficient disease pressure to discriminate effectively between treatments. Plot size however is usually small, given the constraints of mass production of inocula.

The approach taken in this project was to inoculate the two pathogens separately in a protected environment to create uniform disease pressure and allow specific evaluation of different sets of products aimed at the different pathogens.

Methods

A soil-bedded glasshouse at NIAB, Cambridge was used to carry out the experiments. Soil was steam sterilized before the tests started and samples taken for nutrient analysis. Inoculum of each disease was increased and incorporated into the soil before drilling. The crop was grown to the six to seven leaf stage and then assessed for final plant count, fresh harvest weight, and disease as follows:

a) Total plant number in 0.5 m^2 per plot
b) Total fresh weight in 0.5 m^2 per plot
c) Disease symptoms on a sub-sample of 50 plants per plot, taken at random from the harvested areas. Plants were assigned to a disease category on a 0-3 scale for each disease, which was then converted to a disease index using the formula below to convert data to a 0-100 scale:

(0*a+1*b+2*c+3*d)/50 *(100/3), where a,b,c and d are the numbers of plants in each category.

There were eight test products, including one biological, in the *Pythium* trial, and eight in the *Rhizoctonia* trial, including two biologicals. Test products were selected and applied after discussions with AHDB and providers. Most were applied with an AZO compressed air sprayer, but some with a high water volume required were applied with a hand pumped sprayers.

Results

Results from the two trials are presented as mean values with statistical summary in the tables below.

1) *Pythium* test

	Plant count/0.5m²	**Plant weight g/0.5m²**	**Disease index (0-100)**
Treatment			
Untreated	96	941	59.6
Previcur Energy	140	1272	33.4
AHDB9882	114	1259	26.4
AHDB9834	117	1128	22.4
AHDB9883	107	1043	28.4
AHDB9835	124	1101	39.9
AHDB9896	75	934	45.4
AHDB9836	81	938	42.3
AHDB9837	97	985	29.1
	Not significantly different from untreated control (p>0.05)		
	Significantly different from untreated control (p<0.05)		

2) Rhizoctonia test

	Plant count/0.5m²	**Plant weight g/0.5m²**	**Disease index (0-100)**
Treatment			
Untreated	63	470	79.4
Switch	94	817	41.2
Luna Sensation	97	1158	21.3
AHDB9892	76	925	28.7
AHDB9926	93	825	49.6
AHDB9853	126	1159	20.7
AHDB9896	59	571	68.2
T34	61	656	57.1
Amylo X	87	744	56.4
	Not significantly different from untreated control (p>0.05)		
	Significantly different from untreated control (p<0.05)		

Conclusions

Both diseases developed successfully in the trials. Symptoms were distinct, and there was no evidence of cross infection between the trial areas based on visual symptoms. Only one product, which was included in both trials (AHDB9896) showed evidence of potential phytotoxicity, manifested as low plant numbers, though this may also reflect lack of efficacy. In the *Pythium* trial, conventional products performed as well as or close to, the standard over all the harvest measurements. The biological product did not improve plant number or harvest weight, but did significantly reduce disease levels on harvested plants. In the *Rhizoctonia* trial, all the products except one significantly reduced disease symptoms at harvest. One conventional product performed as well as the standard for both disease reduction, plant number, and fresh weight at harvest. The biological products did not significantly improve plant number and fresh weight.

Take home message:

Several conventional products tested merit further investigation. In the *Pythium* trial AHDB numbers 9882, 9834, 9883, and 9837 reduced disease to just below the level of Previcur Energy treatment. In the *Rhizoctonia* trial AHDB9892 and AHDB9853 reduced disease to the same level as Luna Sensation. Biological products also reduced both diseases, though to a lesser extent.

Objectives

1. To establish an effective test system using inoculation of soil-borne diseases in a protected cropping system
2. To generate efficacy data for potential products for *Pythium ultimum* and *Rhizoctonia solani* control in protected cropping of baby leaf salads.
3. To evaluate products for phytotoxicity and effects on plant survival and fresh weight

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
PP 1/148 (2)	Soil treatments against *Pythium* species	Yes

EPPO guidelines for *Pythium* species relate to pot experiments, but in this case, a test more representative of a protected growing system was used. 4 replications were used for conventionals and 6 for biologicals due to constraints on space, the guideline suggested 5. Also the EPPO guideline suggests infected/non-infected assessments only, but in this work a more detailed severity system was used to give a more detailed assessment, and plant survival and fresh weights per unit area were also assessed to support information of product performance.There is no EPPO guideline relating to soil-borne rhizoctonia on protected salad crops.

Test site

Item	**Details**
Location address	NIAB, Park Farm, Villa Road, Histon, Cambridge, CB24 9NZ
Crop	Baby leaf spinach
Cultivar	Aztec FI
Soil or substrate type	Soil – light loam
Agronomic practice	Protected cropping. No herbicides or nutrient feeds required
Prior history of site	Soil bedded glasshouse used for a wide range of crops (ornamental and arable), and steam sterilized before the test was started

Trial design

Item	**Details**
Trial design:	Randomized block
Number of replicates:	X 4 conventional products, X 6 biological
Row spacing:	Not applicable
Plot size: (w x l)	2m x 1m
Plot size: (m^2)	$2m^2$
Number of plants per plot:	Average 200-280/m^2 (in plots treated with standards)
Leaf Wall Area calculations	Not applicable

Inoculation methods and trial maintenance

An isolate of *Pythium ultimum*, originally obtained from a diseased sugar beet seedling, was grown on potato dextrose agar plates. Pearl millet (obtained from a local health food store) was mixed with distilled water (1 kg millet to 250 ml water) in autoclave bags, and then autoclaved at 121°C for 30 minutes on two successive days. Small discs of mycelium *circa* 1 cm in diameter were added to the bags (25 pieces per bag), then the bags were sealed, allowing a small opening for ventilation, and incubated at room temperature in the dark for 3 weeks. Bags were then opened, and the millet seed plus mycelium broken up carefully, spread onto large shallow trays and allowed to dry for 24 h. Any remaining clumps were broken apart and the inoculum divided equally into clean bags for distribution onto the plot area. After application, the millet grains were lightly raked in to the top 5 cm of soil. The amount of inoculum used gave a 1% volume/volume concentration. Pre-tests in a growth room had indicated this would reduce emergence of spinach by approximately 50 %.

Rhizoctonia solani (AG 4), originally isolated from lettuce, was grown on potato dextrose agar plates. A substrate of maizemeal and vermiculite (26 g maizemeal to 111g vermiculite with 200 ml of water) was autoclaved (121°C, 60 minutes) and inoculated with agar discs as for *Pythium*. Bags were incubated at room temperature under natural daylight for 3 weeks. Inoculum was then crumbled, spread out to dry for 48 h, turning from time to time, then measured volumes divided into bags before being scattered over the plot area and raked in as for *Pythium*. A 1% volume/volume incorporation rate was again used as this had produced typical symptoms on all plants (20) used in a pre-test in a controlled environment room.

Inoculation took place on 20/09/19. Soil was then watered periodically to encourage pathogen growth from the inoculation substrates, but was allowed to dry sufficiently so that drilling could take place seven days later on 27/09/19. The area was drilled with a baby leaf spinach variety (Aztec F1) with a grass seed drill pulled by a small tractor through the glasshouse, at seed rate equivalent to 8 million seeds/ha at a depth of 2 cm. Care was taken to avoid moving soil either side of a central pathway of 0.3m. The drill was cleaned between pathogen areas.

The area was watered by overhead sprinkler and daylight supplemented by sodium lights to maintain a 14 h day. Soil analysis showed that SNS was slightly above that recommended for baby leaf production, and P, K and Mg were at the level required, thus no further nutrients were applied to the crop.

Treatment details – *Pythium* test

AHDB Code	Active substance	Product name/ manufacturers code	Formulation batch number	Content of active substance in product	Formulation type	Adjuvant
Untreated						
-	propamocarb hydrochloride+ fosetyl aluminium	Previcur Energy	N/D	530.0g/L propamocarb and 310.0 g/L fosetyl	SC	n/a
AHDB9882	N/D	N/D	N/D	N/D	N/D	n/a
AHDB9834	N/D	N/D	N/D	N/D	N/D	n/a
AHDB9883	N/D	N/D	N/D	N/D	N/D	n/a
AHDB9835	N/D	N/D	N/D	N/D	N/D	
AHDB9896	N/D	N/D	N/D	N/D	N/D	n/a
AHDB9836	N/D	N/D	N/D	N/D	N/D	
AHDB9837	N/D	N/D	N/D	N/D	N/D	n/a

Application schedule – *Pythium* 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	Previcur Energy	15900 g + 9300 g	3ml/m^2 (30l/ha)	A, B
3	AHDB9882	100 g + 1000 g	1.6 l/ha	C
4	AHDB9834	20 g and 250 g	0.2 l/ha mixed with 1l/ha	D
5	AHDB9883	80 g	0.5 l/ha	E
6	AHDB9835	1539 g	4.5 l/ha	F,G
7	AHDB9896	9000 g	20 kg/ha	H
8	AHDB9836	3 x 10^8 cfus	0.3 kg/ha	I,J
9	AHDB9837	187.5 g +750 g	2.5 l/ha	K

Application details – *Pythium* trial

	Application A	Application B	Application C	Application D
Application date	27/09/10	04/10/19	17/10/19	04/10/19
Time of day	09.00-11.00	09.00-11.00	09.00-11.00	09.00-11.00
Crop growth stage (Max, min average BBCH)	00	09	12	09
Crop height (cm)	-	2	15	2
Crop coverage (%)	-	-	>90	
Application Method	drench	drench	spray	spray
Application Placement	soil	soil	foliar	soil
Application equipment	Hozelock Plus	Hoselock Plus	AZO	AZO
Nozzle pressure	N/A	N/A	2.5 bar	2.5 bar
Nozzle type	N/A	N/A	Flat fan	Flat fan
Nozzle size	N/A	N/A	Green GA110-015AZ	Lilac GA110-025AZ
Application water volume/ha	30000 l	30000 l	200 l	300 l
Temperature of air - shade (°C) (figures are air temp in glasshouse)	17.5	16.8	20.0	16.8
Relative humidity (%)	N/A	N/A	N/A	N/A
Wind speed range (m/s)	N/A	N/A	N/A	N/A
Dew presence (Y/N)	N/A	N/A	N/A	N/A
Temperature of soil - 2-5 cm (°C)	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

Application details – *Pythium* trial

	Application E	Application F	Application G	Application H
Application date	27/09/19	04/10/19	17/10/19	27/09/19
Time of day	09.00-11.00	09.00-11.00	09.00-11.00	16.00-18.00
Crop growth stage (Max, min average BBCH)	0	09	12	0
Crop height (cm)	0	2	15	0
Crop coverage (%)	N/A	>90	>90	N/A
Application Method	drench	spray	spray	granule
Application Placement	soil	soil	foliar	soil
Application equipment	AZO	AZO	AZO	N/A
Nozzle pressure	2.5 bar	2.5 bar	2.5 bar	N/A
Nozzle type	Flat fan	Flat fan	Flat fan	N/A
Nozzle size	Lilac GA110-025AZ	Lilac GA110-025AZ	Lilac GA110-025AZ	Fine rose watering can

Application water volume/ha	300 l	300 l	300 l	4 x 20,000 l at 0.5h intervals
Temperature of air - shade (°C) (figures are air temp in glasshouse)	17.5	16.8	20.0	20.2
Relative humidity (%)	N/A	N/A	N/A	N/A
Wind speed range (m/s)	N/A	N/A	N/A	N/A
Dew presence (Y/N)	N/A	N/A	N/A	N/A
Temperature of soil - 2-5 cm (°C)	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

Application details – *Pythium* trial

	Application I	Application J	Application K
Application date	27/09/19	07/10/19	04/10/19
Time of day	13.00-16.00	13.00-16.00	09.00-11.00
Crop growth stage (average BBCH)	0	10	09
Crop height (cm)	0	4	2
Crop coverage (%)	0	>90	>90
Application Method	drench	drench	drench
Application Placement	soil	soil	soil
Application equipment	Kingfisher PS1000	Kingfisher PS 1000	AZO
Nozzle pressure	N/A	N/A	2.5 bar
Nozzle type	N/A	N/A	Flat fan
Nozzle size	N/A	N/A	Lilac GA110-

			025AZ
Application water volume/ha	400 l	400 l	300 l
Temperature of air - shade (°C) (figures are air temp in the glasshouse)	20.2	21.8	16.8
Relative humidity (%)	N/A	N/A	N/A
Wind speed range (m/s)	N/A	N/A	N/A
Dew presence (Y/N)	N/A	N/A	N/A
Temperature of soil - 2-5 cm (°C)	N/A	N/A	N/A
Wetness of soil - 2-5 cm	N/A	N/A	N/A
Cloud cover (%)	N/A	N/A	N/A

Treatment details – *Rhizoctonia* test

AHDB Code	Active substance	Product name/ manufacturers code	Formulation batch number	Content of active substance in product	Formulation type	Adjuvant
Untreated						
Switch	cyprodinil+ fludioxinil	Switch	N/D	37.5% w/w cyprodinil + 25% w/w fludioxonil	WG	n/a
Luna Sensation	fluopyram+ trifloxsytrobin	Luna Sensation	N/D	250g/l fluopyram + 250 g/l trifloxystrobin	SC	n/a
AHDB9892	N/D	N/D	N/D	N/D	N/D	n/a
AHDB9926	N/D	N/D	N/D	N/D	N/D	n/a
AHDB9853	N/D	N/D	N/D	N/D	N/D	n/a
AHDB9896	N/D	N/D	N/D	N/D	N/D	n/a
T34	Trichoderma asperellum	T34	04191830	1 x10^{12} cfu/kg dry weight (12% (w/w)	WP	n/a
Amylo X	Bacillus amyloliquefaciens	Amylo X	N/D	250g/kg Bacillus amyloliquefaciens	WG	n/a

Application schedule – *Rhizoctonia* 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	Switch	300 g + 200 g	0.8 kg	A
3	Luna Sensation	200 g+ 200 g	0.8 l	B
4	AHDB9892	60 g + 90 g	1.2 l	C
5	AHDB9926	400 g	1.0 l	D
6	AHDB9853	50 g	0.5 l	E
7	AHDB9896	9000 g	20 kg	F
8	T34	600 g (5x10^{12} cfus)	5 kg	G
9	Amylo X	625 g	2.5 kg	H,I,J,K

Application details – *Rhizoctonia* test

	Application A	Application B	Application C	Application D
Application date	04/10/19	22/10/19	17/10/19	17/10/19
Time of day	09.00-11.00	09.00-11.00	09.00-11.00	09.00-11.00

Crop growth stage (average BBCH)	09	13	12	12
Crop height (cm)	2	18	15	15
Crop coverage (%)	>90	>90	>90%	>90%
Application Method	spray	spray	spray	spray
Application Placement	soil	foliar	soil+foliar	soil+foliar
Application equipment	AZO	AZO	AZO	AZO
Nozzle pressure	2.5 bar	2.5 bar	2.5 bar	2.5 bar
Nozzle type	Flat fan	Flat fan	Flat fan	Flat fan
Nozzle size	Lilac GA1100-25AZ	Yellow GA110-02AZ	Green GA110-015AZ	Green GA110-015AZ
Application water volume/ha	300 l	500 l (2x250 l)	200 l	200 l
Temperature of air - shade (°C) (figures are air temp in glasshouse)	16.8	18.2	20.0	20.0
Relative humidity (%)	N/A	N/A	N/A	N/A
Wind speed range (m/s)	N/A	N/A	N/A	N/A
Dew presence (Y/N)	N/A	N/A	N/A	N/A
Temperature of soil - 2-5 cm (°C)	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

Application details – *Rhizoctonia* test

	Application E	Application F	Application G	Application H
Application date	04/10/19	27/09/19	27/09/19	04/10/19
Time of day	09.00-11.00	16.00-18.00	16.00-18.00	09.00-11.00

Crop growth stage (average BBCH)	09	0	0	09
Crop height (cm)	2	0	0	2
Crop coverage (%)	>90	N/A	N/A	>90
Application Method	spray	granule	drench	spray
Application Placement	soil	soil	soil	Soil
Application equipment	AZO	N/A	Kingfisher PS 1000	AZO
Nozzle pressure	2.5 bar	N/A	N/A	2.5 bar
Nozzle type	Flat fan	N/A	N/A	Flat fan
Nozzle size	Lilac GA110-025AZ	Fine rose watering can	N/A	Lilac GA110-025AZ
Application water volume/ha	300 l	4 x 20,000 l at 0.5h intervals	2000 l	300 l
Temperature of air - shade (°C) (figures are air temp in glasshouse)	16.8	20.2	20.2	16.8
Relative humidity (%)	N/A	N/A	N/A	N/A
Wind speed range (m/s)	N/A	N/A	N/A	N/A
Dew presence (Y/N)	N/A	N/A	N/A	N/A
Temperature of soil - 2-5 cm (°C)	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

Application details – *Rhizoctonia* test

	Application I	Application J	Application K
Application date	11/10/19	17/10/19	23/10/19
Time of day	09.00-11.00	09.00-11.00	09.00-11.00
Crop growth stage (average	11	12	13

BBCH)			
Crop height (cm)	6	15	18
Crop coverage (%)	>90	>90%	>90%
Application Method	spray	spray	spray
Application Placement	soil+ foliar	foliar	foliar
Application equipment	AZO	AZO	AZO
Nozzle pressure	2.5 bar	2.5 bar	2.5 bar
Nozzle type	Flat fan	Flat fan	Flat fan
Nozzle size	Lilac GA110-025AZ	Lilac GA110-025AZ	Lilac GA110-025AZ
Application water volume/ha	300 l	300 l	300 l
Temperature of air - shade (°C) (figures are air temp in glasshouse)	19.5	20.0	20.7
Relative humidity (%)	N/A	N/A	N/A
Wind speed range (m/s)	N/A	N/A	N/A
Dew presence (Y/N)	N/A	N/A	N/A
Temperature of soil - 2-5 cm (°C)	N/A	N/A	N/A
Wetness of soil - 2-5 cm	N/A	N/A	N/A
Cloud cover (%)	N/A	N/A	N/A

Relative humidity, soil wetness and soil temperature readings were not available on the glasshouse monitoring system. Cloud cover, dew presence and wind speed were not applicable for protected cropping.

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
Damping off	*Pythium ultimum*	PYTHUL	none visible	not applicable	high
Root rot	*Rhizoctonia solani*	RHIZSO	none visible	not applicable	high

Assessment details

Evaluation date		**Days after sowing**	**Crop Growth Stage (BBCH)**	**Evaluation type (efficacy, phytotox)**	**Assessment**
11/10/19		14	11	Phytotoxicity	Foliar symptoms
30/10/19		33	17	Phytotoxicity	Foliar symptoms
04/11/19		38	17	Efficacy	Disease indices on 50

to 07/11/19					plants
04/11/19 to 07/11/19			17	Efficacy	Plant counts per unit area
04/11/19 to 07/11/19			17	Efficacy	Plant fresh weight per unit area

Phytotoxicity symptoms were checked at every trial visit, and formally noted on the dates above. Irrigation was halted two days before assessments began. Assessment of the *Pythium* trial was carried out over 04/11/19 to 05/11/19 and the *Rhizoctonia* trial all on 07/11/19

Unit area was 0.5m^2 per plot, taking care to locate assessment area centrally and avoid possible edge effects.

Scoring scales for the Pythium trial were: 0 – white roots, no rotting, 1 – roots browning, still intact, 2 – roots browning, root thin, no laterals, 3 – root disintegrated, and for the Rhizoctonia trial: 0 = white stem base and roots, 1- slightly brown root, <20% browning at stem base, 2- brown root, 20-50% browning at stem base, 3 – roots disintegrated, >50% stem base and petioles showing browning

Statistical analysis

Statistical analysis was carried out by NIAB statistics group using ANOVA. A missing plot programme was used to account for the unequal replication between conventional products (four) and biologicals (six) to generate adjusted means. Lack of space precluded using six replicates for all products.

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Results

Phytotoxicity

No typical phytotoxicity such as scorching, chlorosis, necrosis or malformation was recorded for any treatment. One product (AHDB9896) resulted in high plant loss compared to untreated at harvest but did give a small reduction (non-significant) in disease index. The product had to be applied with a very high water volume, and it is possible that this resulted in plant loss rather than phytotoxicity *per se*, either through encouraging damping off or creating adverse root growing conditions.

Efficacy

The inoculation technique produced relatively high disease pressure uniformly over the test area so that statistically significant differences between treatments and the untreated plots were obtained for disease severity. Plant counts and harvest weights were more variable, but some significant differences were seen. Symptoms of the two pathogens were distinct, and there was no evidence of cross contamination between the two infected areas.

Results and summary statistics are shown in Table 1 (*Pythium*), and Table 2 (*Rhizoctonia*), and graphically in Figures 1 and 2 respectively for the disease indices in order of increasing severity. All of the products reduced the severity of *Pythium* compared to the untreated plots, though the reduction was not statistically significant for AHDB9896. Four products reduced the disease to slightly less than the level seen in Previcur Energy treated plots, but only one (AHDB9834) gave a harvest weight equivalent to Previcur Energy. In the *Rhizoctonia* trial, two products AHDB9892 and AHDB9853) significantly reduced disease compared to Switch, and gave control at, or near to, the level achieved by Luna Sensation. AHDB9853 was particularly effective in reducing the loss of plants and giving a harvest weight equivalent to Luna Sensation. The two named biocontrol products (T34 and AmyloX) did significantly reduce disease, but to a lesser extent, and did not significantly improve harvest weight.

Table 1 Surviving plant number, plant weight (g) and disease indices, *Pythium* trial.

Product	Plant count/0.5m^2	Plant weight/0.5m^2	Disease index (0-100)
Untreated	96	941	59.6
Previcur Energy	140	1272	33.4
AHDB9882	114	1259	26.4
AHDB9834	117	1128	22.4
AHDB9883	107	1043	28.4
AHDB9835	124	1101	39.9
AHDB9896	75	934	45.4
AHDB9836	81	938	42.3
AHDB9837	97	985	29.1
Trial mean	106	1067	36.3
Standard error	10.1	80.0	5.02
LSD	29.3	234.0	14.59
P value	0.002	0.025	<0.001

Table 2 Surviving plant number, plant weight (g) and disease indices, *Rhizoctonia* trial.

Product	Plant count/0.5m^2	Plant weight/0.5m^2	Disease index (0-100)
Untreated	63	470	79.4
Switch	94	817	41.2
Luna Sensation	97	1158	21.3
AHDB9892	76	925	28.7

AHDB9926	93	825	49.6
AHDB9853	126	1159	20.7
AHDB9896	59	571	68.2
T34	61	656	57.1
Amylo X	87	744	56.4
Trial mean	84	814	47.00
Standard error	9.5	115.8	6.59
LSD	27.4	333.5	19.09
P value	<0.001	0.002	<0.001

Figure 1. Disease indices (0-100) on roots of baby leaf spinach plants from soil inoculated with *Pythium ultimum.*

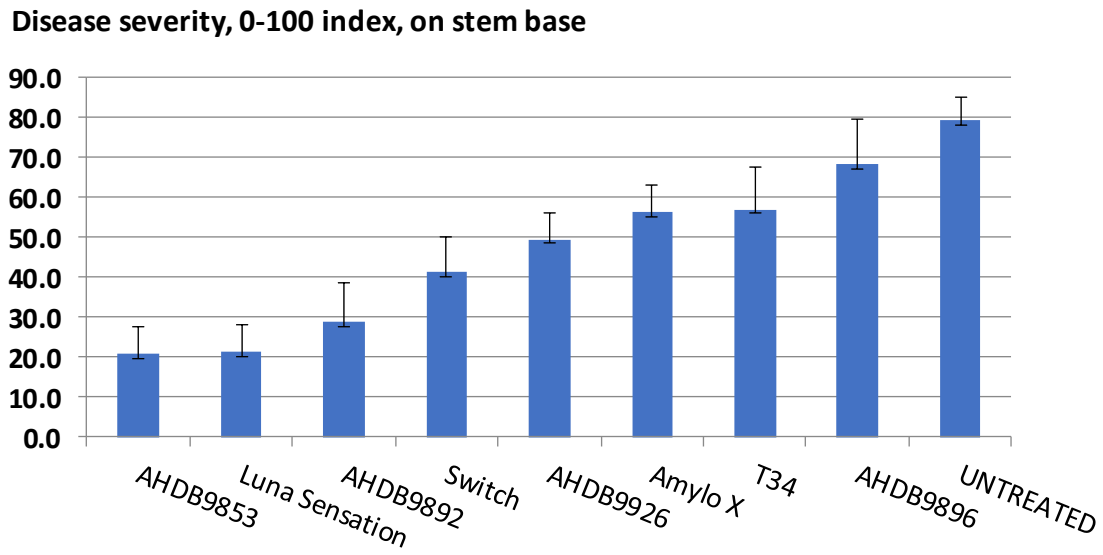

Figure 1. Disease indices (0-100) on stem bases of baby leaf spinach plants from soil inoculated with *Rhizoctonia solani*

Percent reduction in disease indices compared to untreated for *Pythium* is shown in Table 3, and in Table 4 for *Rhizoctonia*

Table 3 Efficacy of products expressed as percent reduction in disease index, *Pythium* trial

Untreated	-
Previcur Energy	43.9
AHDB9882	55.7
AHDB9834	62.4
AHDB9883	52.3
AHDB9835	33.1
AHDB9896	23.8
AHDB9836	29.0
AHDB9837	51.2

Table 4 Efficacy of products expressed as percent reduction in disease index, *Rhizoctonia* trial

Untreated	-
Switch	48.1
Luna Sensation	73.2
AHDB9892	63.9
AHDB9926	37.5
AHDB9853	73.9
AHDB9896	14.1
T34	28.1
Amylo X	29.0

Percent reduction was calculated as:

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

Discussion

The trial was conducted as planned. Non-inoculated check areas were included, but due to restrictions on space, these were located at the end of plot runs close to exit areas, and did not establish well. However, plants appeared healthy, and no disease was recorded in the areas, indicating that inoculum had not moved during the trial duration. The inoculation procedure resulted in high disease pressure throughout each trial. Despite the use of small plots, and the risk of inter-plot interference, most products gave significant reductions in disease. The areas assessed were in the central parts of each plot, allowing for 20-30 cm of discard at the borders where inter-plot interference would have been most likely. Application with an AZO compressed air sprayer was successful for all the products with no blocking problems. Products with very high water volume application rates were however applied with hand pumped sprayers, or in one case, measured volumes from a watering can.

The standard products behaved as expected, thus the trial can be regarded as valid.

Several conventional new products supplied for test in the *Pythium* trial performed close to the standard, and would merit further investigation. The biological product tested also significantly reduced disease, though not to the same extent as some of the conventionals. However, as part of an integrated strategy, the product may have merit, though it was apparent that though disease was reduced, the total plant number and fresh weight did not benefit to the same extent as the conventional products. The high efficacy of four products is encouraging as it provides scope for anti-resistance strategies, with one product, AHDB9834 having a new mode of action and currently in use in the UK as a potato blight fungicide

In the *Rhizoctonia* trial, all products except one significantly reduced disease compared to untreated, and one conventional product, AHDB9853, was as good as one of the standards, and also gave equivalent plant counts and plant weight. The availability of this active ingredient for the salads sector would give an effective alternative to Luna Sensation. The biologicals in this trial gave a lower, but still significant, reduction of disease, but did not improve plant counts or weights. Both however may have merit as part of an integrated control system.

Biological products were applied at the start, or during, the trials, and were not investigated as pre-treatments. Applying biologicals well in advance of planting, to allow propagules to increase further in the soil, may prove more effective, though this approach may be confounded by the use of fungicides later in the programme which may affect viability of fungal biologicals.

Conclusions

- Disease developed to a high level in untreated plots in each trial
- Standard products behaved as expected against each target
- All products except one gave significant reductions in disease in the *Pythium* trial, but only one produced plant weights equivalent to the standard
- All products except one in the *Rhizoctonia* trial gave significant reductions in disease, and three of these improved plant weights equivalent to the standard.
- None of the products which reduced disease showed any evidence of phytotoxicity
- A number of products merit further investigation for each target disease, though as expected, no single product was effective against both targets
- In the *Pythium* trial, AHDB numbers 9834, 9882, 9883 and 9837 all merit further investigation in commercial protected cropping, particularly 9834 due to its new mode of action.In the *Rhizoctonia* trial AHDB 9853 and 9892 both merit being taken forward.
- Biological products should be further investigated as advanced pre-treatments, with promising conventional products applied subsequently. Investigation of the survival of biological products in the cropping environment would also be merited to help to formulate procedures for a systems based approach combining the different types of product.

Acknowledgements

Funding from AHDB SceptrePlus is gratefully acknowledged. Thanks are also due to manufacturers for supply of products and helpful discussions, and Tozer Seeds Ltd for supply of untreated spinach seed.

Appendix A

Crop diary

Date	Event
27/09/19	sowing
28/09/19	Irrigation on: 5 minutes at 07.00 and 5 minutes at 18.00*
01/10/19	Crop at 10-20% emergence
02/11/19	Irrigation turned off
04/11/19 to 07/11/19	Plant counts, fresh weight and disease assessments

*irrigation was suspended after afternoon sprays. Crop was dry before morning irrigation

Appendix B Trial diary

Date	Event
19/08/19	Cultures increased on agar plates
27/08/19 - 28/08/19	Inoculum placed in autoclaved substrate bags
18/09/19	Bags opened and inoculum crumbled and air dried
20/09/19	Inoculum applied to test areas and raked in
21/09/19	Irrigation applied
25/09/19	Irrigation stopped to allow drying for drill

Appendix C Trial photographs

Trial inoculation 20/09/19

Trial drilling, 27/09/19

Trial harvest, week beginning 04/11/19

Disease categories Pythium: 0 – white roots, no rotting, 1 – roots browning, still intact, 2 – roots browning, root thin, no laterals, 3 – root disintegrated

Disease categories Rhizoctonia 1- slightly brown root, <20% browning at stem base, 2- brown root, 20-50% browning at stem base, 3 – roots disintegrated, >50% stem base and petioles showing browning (0 as for healthy plant in Pythium picture)

Appendix D Climatalogical data

Date	Max C	Min C	Date	Max C	Min C
20/09/2019	29.7	9.7	13/10/2019	21.2	15.0
21/09/2019	31.5	11.7	14/10/2019	20.1	14.3
22/09/2019	27.1	16.0	15/10/2019	21.9	15.2
23/09/2019	24.4	14.7	16/10/2019	21.1	14.3
24/09/2019	24.1	16.7	17/10/2019	21.5	11.8
25/09/2019	24.6	15.6	18/10/2019	20.9	14.0
26/09/2019	26.7	14.3	19/10/2019	21.5	13.4
27/09/2019	24.7	13.5	20/10/2019	20.4	12.4
28/09/2019	26.8	15.5	21/10/2019	19.5	14.4
29/09/2019	28.3	16.9	22/10/2019	20.9	12.9
30/09/2019	25.9	14.6	23/10/2019	22.1	11.1
01/10/2019	25.6	16.2	24/10/2019	20.4	15.3
02/10/2019	23.4	12.0	25/10/2019	20.7	13.2
03/10/2019	22.2	10.0	26/10/2019	19.7	16.6
04/10/2019	25.4	14.0	27/10/2019	20.4	10.7
05/10/2019	24.4	14.9	28/10/2019	20.7	9.8
06/10/2019	23.9	15.4	29/10/2019	20.8	9.8
07/10/2019	22.3	12.8	30/10/2019	20.5	10.1
08/10/2019	23.8	14.2	31/10/2019	20.2	9.8
09/10/2019	23.1	13.5	01/11/2019	20.5	12.6
10/10/2019	23.5	13.4	02/11/2019	18.5	14.6
11/10/2019	21.3	16.0	03/11/2019	20.0	12.8
12/10/2019	20.5	15.2	04/11/2019	20.4	12.8

Appendix E Raw data

Pythium raw data

Treatment	Plot number	plant count	weight	DI
Untreated	9	100.0	1182.9	52.7
	14	112.0	1241.3	57.3
	23	105.0	959.9	14.7
	29	142.0	1202.7	66.0
	44	55.0	723.8	81.3
	46	62.0	332.4	85.3
Previcur Energy	7	108.0	1469.1	21.3
	15	159.0	1470.7	35.3
	24	134.0	1039.0	26.0
	30	198.0	1557.4	18.7
AHDB9882	6	63.0	1149.4	20.7
	18	137.0	1420.4	22.0
	25	143.0	1366.1	6.7
	32	153.0	1546.4	24.0
AHDB9834	2	84.0	975.6	6.7
	17	154.0	1788.0	10.7
	19	119.0	880.7	18.0
	28	150.0	1316.6	22.0
AHDB9883	3	132.0	1245.5	18.7
	11	105.0	1298.9	11.3
	27	116.0	1098.5	19.3
	35	115.0	975.9	32.0
AHDB9835	4	98.0	1216.7	49.3
	12	144.0	1353.6	25.3
	20	145.0	905.7	25.3
	31	150.0	1376.9	27.3
AHDB9896	8	50.0	797.3	60.0
	16	104.0	1520.3	14.7
	21	86.0	740.7	23.3
	36	98.0	1125.0	51.3
AHDB9836	5	65.0	968.7	39.3
	10	85.0	918.2	42.7
	22	120.0	905.4	27.3
	34	59.0	1035.9	42.7
	43	104.0	1123.3	43.3
	49	53.0	674.1	58.7
AHDB9837	1	108.0	1182.5	8.0
	13	139.0	1058.8	30.0
	26	98.0	1032.9	8.7
	33	82.0	1113.5	37.3

Rhizoctonia raw data

Treament	Plot number	plant count	weight	DI
Untreated	4	87.0	805.0	52.7
	10	53.0	404.5	73.6
	24	35.0	250.5	86.7
	32	99.0	646.8	90.7
	37	42.0	353.4	92.1
	51	60.0	361.7	80.7
Switch	8	85.0	933.6	17.0
	13	71.0	980.5	45.3
	23	97.0	688.2	58.7
	35	127.0	826.5	42.0
Luna Sensation	9	80.0	1262.4	6.7
	15	85.0	872.1	31.9
	26	110.0	1373.6	32.7
	36	117.0	1282.9	12.0
AHDB9892	3	119.0	1142.0	5.3
	17	42.0	738.3	28.6
	19	69.0	683.7	53.1
	34	78.0	1296.7	26.0
AHDB9926	2	124.0	740.5	39.3
	11	79.0	854.7	49.3
	25	86.0	647.2	67.3
	29	90.0	1216.1	40.7
AHDB9853	7	107.0	1128.0	11.3
	12	165.0	1773.0	9.3
	20	110.0	745.9	38.7
	28	128.0	1147.3	21.5
AHDB9896	1	63.0	424.5	57.3
	14	71.0	1204.8	40.7
	22	45.0	332.2	90.4
	30	61.0	480.4	82.6
T34	5	58.0	517.5	59.3
	18	40.0	611.0	67.5
	27	43.0	808.5	28.0
	31	68.0	597.1	86.7
	40	86.0	983.5	25.3
	50	68.0	420.1	76.0
Amylo X	6	105.0	1253.1	39.3
	16	37.0	874.0	52.3
	21	115.0	825.2	44.7
	33	114.0	364.9	85.3
	45	89.0	583.1	56.9
	52	60.0	565.0	60.0

Appendix F Trial design

Randomised block. Rhizoctonia (Left hand side below) and Pythium (right hand side) separated by 0.3m central non-inoculated gap

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