SCEPTREPLUS

Final Trial Report

Trial code:	SP 63
Title:	**Spinach Stemphylium Leaf Spot (seed treatments)**
Crop	Spinach
Target	*Stemphylium botryosum*
Lead researcher:	Dave Kaye
Organisation:	ADAS Horticulture
Period:	2 Years
Report date:	December 2021
Report author:	Catherine Eyre, Callum Burgess
ORETO Number: (certificate should be attached)	ORETO 409

I the undersigned, hereby declare that the work was performed according to the procedures herein described and that this report is an accurate and faithful record of the results obtained

…… ……

Date: 17/03/23 Authors signature

Trial Summary

Introduction:

Seed treatments are an important component of disease control in the early development of leafy green vegetables and can be important in both the quality control and yield of crops. Thiram was the industry standard plant protection product for seeds until its recent withdrawal. Alternative seed treatments are required to prevent disease caused by *Stemphylium botryosum* and other seed borne pathogens.

S. botryosum causes leaf spotting that weakens the plant and reduces the photosynthetic area of the leaf, hindering the ability to grow. Severely affected leaves can have lesions that cause shrivelling and dieback. *S. botryosum* has a short latent period and so once present in a crop can spread quickly. Ascospores of *S. botryosum* grow on older lesions and require moist conditions to form. Once in the seeds *S. botryosum* can be difficult to treat. Preventative measures to inhibit disease growth or presence on seed can be a cost-effective means of averting disease within a crop.

Despite a considerable amount of research into control of *S. botryosum* on seed effective options are not available for treatment of seeds in Great Britain. The objective of this study was to test the efficacy of several new and commercially available fungicides as seed treatments against *S. botryosum* on spinach.

Methods

1. Seed germination trial – inoculated treated seeds placed on filter paper and assessed for germination rate and seedling quality only.
2. Agar plate efficacy trial – inoculated treated seeds plated onto agar to evaluate for presence of *S. botryosum*.

Seed preparation

Trials were conducted with spinach seed of the susceptible variety, Monterey. Seeds were surface sterilized with 1% sodium hypochlorite and dried, before an application of *S. botryosum* spore solution (10^6 spores/ml) was used to coat the seed surface. Seeds were subsampled and treated (10 treatments) (Table 1) by Elsoms Seeds Ltd in October 2021.

1. Germination trial
    - Conducted 1 week post-treatment
    - 200 seeds per treatment – 4 reps of 50
    - Seeds arranged on moist filter paper in closed boxes for 28 days at 20-24^{o}C.
    - Assessed weekly for:
        - Number of germinated seeds
        - Seedling growth stage (BBCH scale leafy vegetables not forming heads)
        - Phytotoxicity (% of plot), i.e. seedling damage, compared with untreated uninoculated. (0-100% scale)
        - Seedling quality assessment (0-4) at final destructive assessment as below:

            4) Germinated with normal seedling development: Cotyledons at least 50% emerged with no damage to terminal bud, roots over 1.0 cm.

            3) Germinated with weak growth, and roots 0.5 – 1.0 cm.
            2) Germinated with abnormal growth (such as twisting, necrotic spots), and roots less than 0.5 cm.
            1) Ungerminated viable seed: Seeds which remain firm and apparently viable at the end of the test.
            0) Ungerminated dead seed: Seeds which at the end of the test period were either decayed, mouldy or soft.

- Fresh weight (g) of germinated seedlings at final assessment per plot (50 seeds sown per plot).
- All measures compared against the untreated uninoculated control.

2. Agar plate efficacy trial

- Randomized block design
- 5 seeds per plate, plated onto potato dextrose agar amended with ampicillin (200 ug/ml), streptomycin (200 ug/ml), and chlortetracycline (20 ug/ml)
- 11 replicate plates for each of 10 treatments, including an untreated uninoculated, untreated inoculated and film coat control. 110 plates total.
- Seeds evaluated for incidence of *S. botyrosum* and other species growth - 5 times in 7 days.
- At final assessment (7 days) fungal species were identified.

Table 1. The treatment numbers, with their associated applications (AHDB codes) showing the rates that were used for each application and the timings at which they were applied. Treatments applied as a seed treatment in October 2021.

Trt. No.	Product	Rate (L/ha or kg/ha)
1	Untreated uninoculated	-
2	Untreated inoculated	-
3	Seed coat inoculated	-
4	Acetic acid	10% solution
5	AHDB9797	120 g per L water as a soak for 10 mins, rinse and airdry
6	Hydrogen Peroxide	3% solution
7	Integral Pro	10 ml / kg seed
8	AHDB9815	5g/kg seed
9	AHDB9847	1.0 ml / kg seed
10	AHDB9848	10 ml / kg seed

Results

1. Germination trial

Seed germination

- Overall spinach seed germination was low even in untreated uninoculated seeds; average germination was 34.5% of 200 seeds (4 reps of 50).
- Apart from the acetic acid treatment, the untreated inoculated seeds had one of the poorest germination rates (8%).
- Acetic acid completely inhibited germination.
- AHDB9848, AHDB9815 and AHDB9797 had the highest seed germination rates of all treatments at the final assessment and were not significantly different from the untreated uninoculated control.
- Apart from acetic acid, AHDB9847, Integral Pro and Hydrogen peroxide were the poorest performers across all time points.

Table 2. Mean percentage seed germination for 10 treatments assessed for three weeks post-sowing. Data reported is from raw assessments but statistical analysis was performed on transformed data.

Treatment	Weeks post sowing			Duncan's multiple range test (3 weeks post sowing)
	1	2	3	
Untreated uninoculated	20.5	21.5	34.5	d
Untreated Inoculated	4.5	7	8	ab
Filmcoat green	14.5	17	19	abcd
Acetic acid	0	0	0	a
AHDB9797	17.5	21	22	bcd
Hydrogen peroxide	3	8	12.5	abc
Integral Pro	9.5	10	11.5	abc
AHDB9815	15.5	20.5	28.5	bcd
AHDB9847	8	10	10	abc
AHDB9848	9.5	19	30.5	cd
p-value	0.001	0.014	0.012	
d.f.	30	30	30	
L.S.D.	9.408	12.562	18.66	
Significantly different from the untreated control (P<0.05)				
Not significantly different from the untreated uninoculated control				

Seedling quality

- The untreated uninoculated seeds had the highest average quality score as expected.
- Generally more seeds were ungerminated than germinated. Of those germinated the untreated uninoculated control, filmcoat green inoculated control and AHDB9815 had the most high-quality plants.
- AHDB9815 had the highest average quality score 1.24. 124 of 200 (62%) seeds were scored as high quality, but 82 of 200 (41%) did not germinate.
- AHDB9847 performed least well with all those emerged seedlings being of low quality.

Fresh weight of germinated seedlings

- Seedlings of AHDB9815 had highest average fresh weight (0.92g). Significantly higher than all other treatments and the untreated uninoculated control.

Phytotoxicity

- Percentage phytotoxicity was assessed for each plot. Observed symptoms included stunted thickened roots, collapsed plants with curled growth, root browning and poor or no lateral root production.
- All controls (inoculated, uninoculated, filmcoat green inoculated), Integral Pro, AHDB9815 and hydrogen peroxide had no seedlings with signs of phytotoxicity.
- Acetic acid scored 100% phytotoxicity with no seeds emerging.
- Average phytotoxicity, observed as damage and deformation of growth, increased over time. Final assessment averages for those treatments affected were AHDB9847 (50%), AHDB9797 (30%) and AHDB9848 (20%)

2. Efficacy of treatment to control pathogen growth from seed

- All treated seeds developed some fungal growth when plated on agar after 7 days, apart from those treated with acetic acid which had just 7% seeds with growth.
- Stemphylium was observed in all treated seeds apart from acetic acid.
- Filmcoat green had a significantly lower percentage of seeds (12.7%) with Stemphylium growth compared to other treatments and was not significantly different from the untreated uninoculated control (3.6%). Filmcoat treated green seeds, however, had the highest percentage infected with Penicillium (83.6%).
- The greatest number of Stemphylium cultures grew from the untreated inoculated control (94.6%), AHDB9847 (98.2%), AHDB9848 (92.7%) and AHDB9797 (92.7%) (Table 3).
- Penicillium, Fusarium, Pythium, Botrytis, some unknowns and yeasts also grew on seeds.

Table 3. Percentage of seeds identified to have each fungal genus growing from them in the agar plating test, 7 days after plating. Eleven plates with 5 seeds each (total 55 seeds) for each treatment. Data reported is from raw assessments but statistical analysis was performed on transformed data.

Treatment	Fungal growths on seeds							
	Stemphylium	Penicillium	Fusarium	Pythium	Botrytis	Unknown	Yeast	No growth
Untreated uninoculated control	3.64	7.27	0.00	0.00	3.64	80.00	5.45	0.00
Untreated inoculated control	94.55	5.45	0.00	0.00	0.00	0.00	0.00	0.00
Filmcoat green	12.73	83.64	3.64	0.00	0.00	0.00	0.00	0.00
Acetic acid	0.00	5.45	0.00	0.00	0.00	1.82	0.00	92.73
AHDB9797	92.73	7.27	0.00	0.00	0.00	0.00	0.00	0.00
Hydrogen peroxide	63.64	21.82	0.00	12.73	0.00	1.82	0.00	0.00
Integral Pro	61.82	32.73	1.82	0.00	0.00	0.00	0.00	3.64
AHDB9815	50.91	49.09	0.00	0.00	0.00	0.00	0.00	0.00
AHDB9847	98.18	1.82	0.00	0.00	0.00	0.00	0.00	0.00
AHDB9848	92.73	3.64	0.00	0.00	0.00	1.82	0.00	1.82
p-value	<.001	<.001						
d.f.	100	100						
L.S.D.	14.32	12.866						
Significantly different from the untreated control								
Not significantly different from the untreated control								

Conclusion

Overall the best performing product was AHDB9815, a biological treatment, which had the highest seed germination rate, plant quality and fresh weight, and no observed phytotoxicity. In the agar plating efficacy test there was some Stemphylium growth but this treatment tended to have fewer seeds affected than other treatments. The other biological product Integral Pro, resulted in poor germination rate, but no phytotoxicity and a high proportion of seeds with Stemphylium growth.

The chemical treatments did not perform well at the rates used. Acetic acid inhibited all Stemphylium growth but also prevented germination completely at the rate used. Hydrogen peroxide resulted in poor germination rates, low quality plants and high number of seeds with Stemphylium growth. AHDB9797 had relatively good seed germination but poor plant quality,

signs of phytotoxicity and Stemphylium detected in numbers comparable to the untreated inoculated control.

AHDB9848 was the best performing product of the two conventional fungicides. It had one of the highest germination rates but showed phytotoxic effects and had a high proportion of seeds with Stemphylium detected. The other conventional fungicide AHDB9847 was one of the worst products across all metrics and does not appear to offer good protection against *S. botryosum*.

Take home message:

No treatments offered full control of *S. botryosum* but the best performer was the biological product, AHDB9815.

Full report:

Summary

Seed treatments are an important component of disease control in the early development of leafy green vegetables and can be important in both the quality control and yield of crops. Thiram was the industry standard plant protection product for seeds until its recent withdrawal. Consequently, alternative seed treatments are required to prevent disease caused by *S. botryosum* and other seed borne pathogens.

S. botryosum causes leaf spotting in spinach that weakens the plant and reduces the photosynthetic area of the leaf, hindering the ability to grow. Severely affected leaves can have lesions that cause shrivelling and dieback. *S. botryosum* has a short latent period and so once present in a crop can spread quickly. Ascospores of *S. botryosum* grow on older lesions and require moist conditions to form. Once in the seeds *S. botryosum* can be difficult to treat. Preventative measures to inhibit disease growth or presence on seed can be a cost effective means of averting disease within a crop.

The objective of this study was to test the efficacy of several new and commercially available fungicides as seed coats against *S. botryosum* on spinach.

Objectives

To evaluate the efficacy (disease incidence and severity) and crop safety of fungicide seed treatments of spinach against Stemphylium (*S. botryosum*) compared to an untreated control:

1. To assess crop safety of seed treatments in spinach.
2. To evaluate impact of seed treatment on pathogen presence.

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**
EPPO PP1/135(4)	Phytotoxicity assessment	N/A
EPPO PP1/152(4)	Guideline on design and analysis of efficacy evaluation trials	N/A
EPPO PP1/225 (2)	Minimum effective dose	N/A
EPPO PP1/181 (4)	Conduct and reporting of efficacy evaluation trials including good experimental practice	N/A
EPPO PP 1/214(3)	Principles of acceptable efficacy	N/A
EPPO PP 1/224(2)	Principles of efficacy evaluation for minor uses	N/A

An experimental permit was required for this work and has been obtained for all the test treatments by AHDB Horticulture as part of the SCEPTREplus programme (AGRON/056, permit numbers COP 2017/01964, 2018/00238, 2018/01906).

Test site

Item	Details
Location address	Battle Gate Rd, Cambridge CB23 4NN
Crop	Spinach
Cultivar	Monterey
Soil or substrate type	PDA + Ampicillin Streptomycin +Chlortetracycline
Agronomic practice	N/A
Prior history of site	N/A

Trial design:

Two trials were conducted as follows:

1. Seed germination trial to assess phytotoxicity
2. Treatment efficacy assessment by determination of pathogen presence on treated seed.

1. Seed germination trial to assess phytotoxicity

Seeds were inoculated with a culture of *Stemphylium botryosum* and then product treatments applied. 1 week after treatment a germination test on filter paper was conducted to assess treatment phytotoxicity as described below.

Item	Details
Trial design:	Randomized block
Number of replicates:	4 germination trial
Row spacing:	N/A
Plot size: (w x l)	15cm for germination trial
Plot size: (m^2)	N/A
Number of plants per plot:	50 seeds per tub for germination trial
Leaf Wall Area calculations	N/A

2. Treatment efficacy assessment – pathogen presence on seed

Treated seed was plated onto agar and evaluated for growth of stemphyllium from seeds as described below.

Item	Details
Trial design:	Randomized block
Number of replicates:	11 for disease trial
Row spacing:	N/A
Plot size: (w x l)	90mm for disease trial
Plot size: (m^2)	N/A
Number of plants per plot:	5 seeds per plate for disease trial
Leaf Wall Area calculations	N/A

Treatment details

AHDB Code	Content of active substance in product	Formulation type
Untreated uninoculated	N/A	N/A
Untreated inoculated	N/A	N/A
Seed coat inoculated	N/A	N/A
Acetic acid	100%	flowable concentrate
AHDB9797	100%	Wettable powder
Hydrogen Peroxide	3%	flowable concentrate
Integral Pro	6.8% w/w	Flowable concentrate
AHDB9815	17,5% p/p (1x 10^6 ufc/g)	flowable concentrate
AHDB9847	100g/l	Flowable concentrate
AHDB9848	200 g/L 150 g/L	Flowable concentrate

Methods, assessments and records

Product efficacy and crop safety experiments were conducted with spinach seed of the susceptible variety Monterey. Seeds were inoculated with *S. botryosum* and then treated with 7 different crop protection products by Elsoms Seeds Ltd. All seeds also received Filmcoat green which is a standard commercial practice. A seed batch with sufficient natural inoculum was not available, therefore a method for artificial seed inoculation had to be developed within this study.

Seed preparation

Surface sterilization
Spinach seeds were soaked in a 1% sodium hypochlorite solution for 30 seconds, followed by three 1 minute rinses in sterile distilled water and then air dried in a laminar flow hood. Seed was stored in the dark under cool (ca. 5°C), dry conditions until required.

Sampling
Seeds were sampled randomly to avoid any bias towards a particular seed size, shape, density or other quality trait. 50 g of seeds was sampled for each treatment, including controls. Seed was stored in paper bags, stored under dark, cold (ca. 5°C), dry conditions before use.

Inoculum preparation
Cultures of *S. botryosum* were taken from the ADAS culture collection and pathogenicity confirmed by inoculation of spinach leaves. Cultures grown on PDA agar at 20°C for 10 days or until spores were visible. Spores were harvested by adding 5-10ml of sterile distilled water to each plate and a sterile spreader used to dislodge spores. The resulting spore suspension from plates was drawn off and pooled from multiples plates. The spore suspension was passed through 4 layers of muslin cloth and then spore counts made using a

haemocytometer. The suspension was diluted to a final concentration of $1x10^6$ spores/ml using sterile distilled water.

Seed inoculation

Surface sterilized seed was soaked for 4 hours in the *S. botryosum* spore solution. The mixture was periodically stirred to ensure even distribution. The coated seeds were spread onto paper towel in a laminar flow and allowed to dry for 3 days at room temperature (approx. 20°C).

Seed treatment application

Elsoms Seeds Ltd. applied the product treatments using a commercial seed treatment facility according to standard in-house protocols for small batches of seed. Briefly, the seed was weighed and treatments applied at the required rates using a pipettor in a moving rotary drum (desktop treater – Hoopman). Polymer (Seedcoat Green) was applied at the advised rates via syringe and the same rotary disc and drum method. Seed was removed from the drum and placed into muslin bags before being dried at 38°C in a pelleting drier for 10 minutes, or until the seed was at an acceptable level of relative humidity.

Application schedule

Treatment number	Treatment: product name or AHDB code	Rate of product (l or kg/ha)	Content of active substance in product	Rate of active substance (ml or g a.s./ha)	Application code
1	Untreated uninoculated	N/A	N/A	N/A	N/A
2	Untreated inoculated	N/A	N/A	N/A	N/A
3	Seed coat inoculated	N/A	N/A	N/A	N/A
4	Acetic acid	10% solution		10%	A
5	AHDB9797	120 g per L water as a soak for 10 mins, rinse and airdry.		N/A	A
6	Hydrogen Peroxide	3% solution		30 ml/L	A
7	Integral Pro	10 ml / Kg seed	2.2×10^{10} cfu/mL (6.12% w/w)	2.2 x 10^11 cfu/kg 0.612 g/kg	A
8	AHDB9815	5g/kg seed	17.500% w/w pythium oligandrum M1	N/A	A
9	AHDB9847	1.0 ml / Kg seed	100g/l prothioconazole	N/A	A
10	AHDB9848	10 ml / Kg seed	200 g/L fluopicolide and 150 g/L fluoxastrobin	N/A	A

Application details

Application date	Application A
Time of day	N/A
Crop growth stage (Max, min average BBCH)	Seed
Crop height (cm)	0
Crop coverage (%)	N/A
Application Method	Varied
Application Placement	Seed
Application equipment	Varied
Nozzle pressure	N/A
Nozzle type	N/A
Nozzle size	N/A
Application water volume/ha	N/A
Temperature of air - shade (°C)	Indoors
Relative humidity (%)	N/A
Wind speed range (m/s)	N/A
Dew presence (Y/N)	N
Temperature of soil - 2-5 cm (°C)	N/A
Wetness of soil - 2-5 cm	N/A
Cloud cover (%)	N/A

Common name	**Scientific Name**	**EPPO Code**	Infestation level at start of assessment period	**Infestation level In the middle of assessment period**	**Infestation level at end of assessment period**
Stemphylium	N/A	N/A	$1*10^6$	N/A	N/A

1. Germination trial

Spinach seeds were inoculated with *S. botryosum* at a rate of 1 x 10^6 spores/ml as described above and then sent for application of treatments by Elsoms Seeds Ltd. 1 week after seed treatments were applied the germination trial was set up.

Each treated seed batch was subsampled for 200 seeds. Moist filter papers were used to line plastic trays and these seeds were sown in a 10 x 5 grid, with four reps of 50 for each treatment. Trays were covered with lids to prevent moisture loss and incubated in a CE cabinet at 20ºC with a 16:8 hour light:dark cycle for 21 days. Boxes were checked every 2-3 days to ensure the filter paper remained moist.

Seed germination was assessed every 7 days up to 28 days for the following metrics, with the final assessment being destructive.

- Number of germinated seeds
- Seedling growth stage – according to BBCH growth stage scale (Figure 1, Appendix A)
- Phytotoxicity (%) – Scored as percentage seedlings per plot affected with symptoms of phytotoxicity irrespective of severity. Conducted every week for three weeks from first sign of germination.

- Plant quality (0-4 score) – at final assessment.
    - 4 - Germinated with normal development: Cotyledons at least 50% emerged with no damage to terminal bud, roots over 1.0 cm.
    - 3 - Germinated with weak growth, and roots 0.5 – 1.0 cm.
    - 2 - Germinated with abnormal growth (such as twisting, necrotic spots), and roots less than 0.5 cm.
    - 1 - Ungerminated viable seed: Seeds which remain firm and apparently viable at the end of the test.
    - 0 - Ungerminated dead seed: Seeds which at the end of the test period were either decayed, mouldy or soft.

- Fresh weight (g) of germinated seedlings harvested from whole plot at final assessment (50 seeds sown per plot).
- Observations on growth - include stunting of growth, discoloration of leaves and roots, chlorosis, spotting, necrosis, twisting, crinkling, leaf, or root thickening, amongst other effects.

2. Treatment efficacy assessment – pathogen presence on seed

Freeze blotter assays are often used to detect fungi in seeds. The seed is frozen to make it unviable to prevent germination and then ungerminated seeds are incubated in ideal fungal growth conditions and evaluated for growth of the target pathogen. Preliminary work trialed this type of assay to determine the efficacy of seed treatments in inhibiting pathogen growth from seeds. The initial trials found that sporulation of Stemphylium did not occur quickly to allow identification, and contaminants quickly overgrew seeds masking any later sporulation of Stemphylium that might be detected.

An alternative experimental method was trialed and eventually used in this study, where inoculated treated seeds were plated onto potato dextrose agar amended with antibiotics (Chlortetracycline 20ug/ml, Ampicillin 200ug/ml, Streptomycin 200ug/ml). Plates were incubated at 20^{o}C for 7 days. Colonies growing out from seeds onto agar were identified and assessed. Allowing the cultures to grow on agar allowed enough visible mycelium to be present to accurately identify *Stemphylium* cultures.

Seeds were monitored daily and the number of seeds with visual fungal growth counted 4 times in a 7-day period. After 7 days, when identifiable features had developed the fungal colonies were identified to genus level by microscopy. At this final assessment those seeds with *S. botryosum* cultures were identified.

Table 4. Assessment dates for agar seed trial

Evaluation date	Days after seed plating	Crop Growth Stage (BBCH)	Evaluation type (efficacy, disease prevention)	Assessment
16/11/21	1	N/A	Disease count	Disease presence 1
17/11/21	2	N/A	Disease count	Disease presence 2
18/11/21	3	N/A	Disease count	Disease presence 3
19/11/21	4	N/A	Disease count	Disease presence 4
22/11/21	7	N/A	Disease count	Disease presence 5

Statistical analysis:

The germination tests and efficacy trial were laid out as a randomised complete block design. Statistical analysis was conducted using Genstat 18. Fresh weight (g) was analysed using ANOVA with a Duncan's Multiple Range Test. Percentage data for other measures (germination, plant quality, fungal growth on seed and fungal identification on seed) was

transformed using an angular transformation and analysed with ANOVA with a Duncan's Multiple Range Test. Data reported in the tables and figures are the raw means, but the statistical analysis was conducted on the transformed data. All treatments were compared with the untreated uninoculated control.

Results

1. Germination trial

Seed germination

Average germination for the untreated uninoculated control at the last assessment, 3 weeks post-sowing, was 34.5%. This was lower than would be expected for a normal healthy seed batch. The untreated inoculated seeds had the lowest average germination (8%). Seeds with treatments applied had final germination averages that fell between the uninoculated untreated control and the inoculated untreated seeds (range of 10-30.5%), with the exception of acetic acid where the treatment appeared to completely kill seeds with zero germination (Figure 1, Table 5). At the last assessment, the filmcoat green and AHDB9797, AHDB9815 and AHDB9848 treatments were not significantly different from the untreated uninoculated control. The remaining treatments acetic acid, hydrogen peroxide, Integral Pro and AHDB9847 were not significantly different from the untreated inoculated control (Duncan's multiple range test, Table 5).

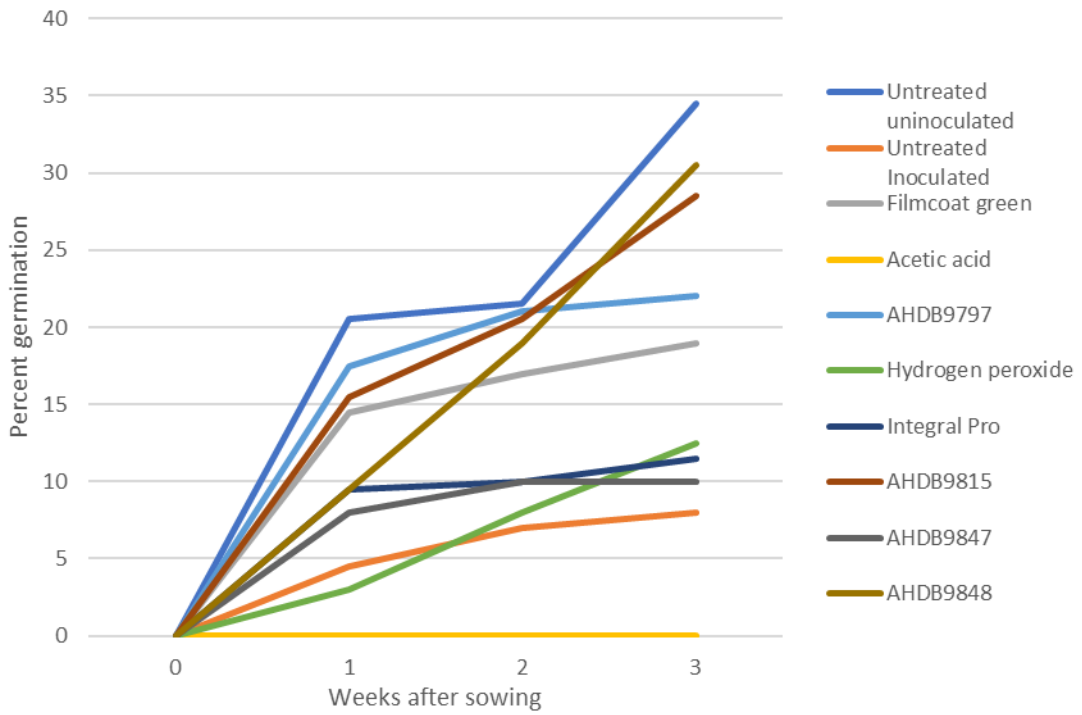


Figure 1. Mean percentage seed germination for 200 seeds (4 reps of 50) with 10 treatments for 3 weekly assessments post-sowing.

Table 5. Mean percentage seed germination for 10 treatments assessed for three weeks post-sowing. Data reported is from raw assessments but statistical analysis was performed on transformed data.

Treatment	Weeks post sowing			Duncan's multiple range test (3 weeks post sowing)
	1	2	3	
Untreated uninoculated	20.5	21.5	34.5	d
Untreated Inoculated	4.5	7	8	ab
Filmcoat green	14.5	17	19	abcd
Acetic acid	0	0	0	a
AHDB9797	17.5	21	22	bcd
Hydrogen peroxide	3	8	12.5	abc
Integral Pro	9.5	10	11.5	abc
AHDB9815	15.5	20.5	28.5	bcd
AHDB9847	8	10	10	abc
AHDB9848	9.5	19	30.5	cd
p-value	0.001	0.014	0.012	
d.f.	30	30	30	
L.S.D.	9.408	12.562	18.66	
Significantly different from the untreated control (P<0.05)				
Not significantly different from the untreated uninoculated control				

Plant quality

Untreated uninoculated seeds had the highest percentage of seeds (28%) in the high-quality category, although this was lower than might be expected from this control. The high-quality plants maintained good colour, root length, lateral root growth, foliar size and stem length. All treatments and the inoculated control had significantly reduced percentages of high-quality plants (Table 6). For all treatments the majority of seedlings fell into the ungerminated seed categories (viable and unviable) (Figure 2, Table 6). The treatments with the highest percentage of high quality were AHDB9815 (15.5%) and filmcoat green (16%).

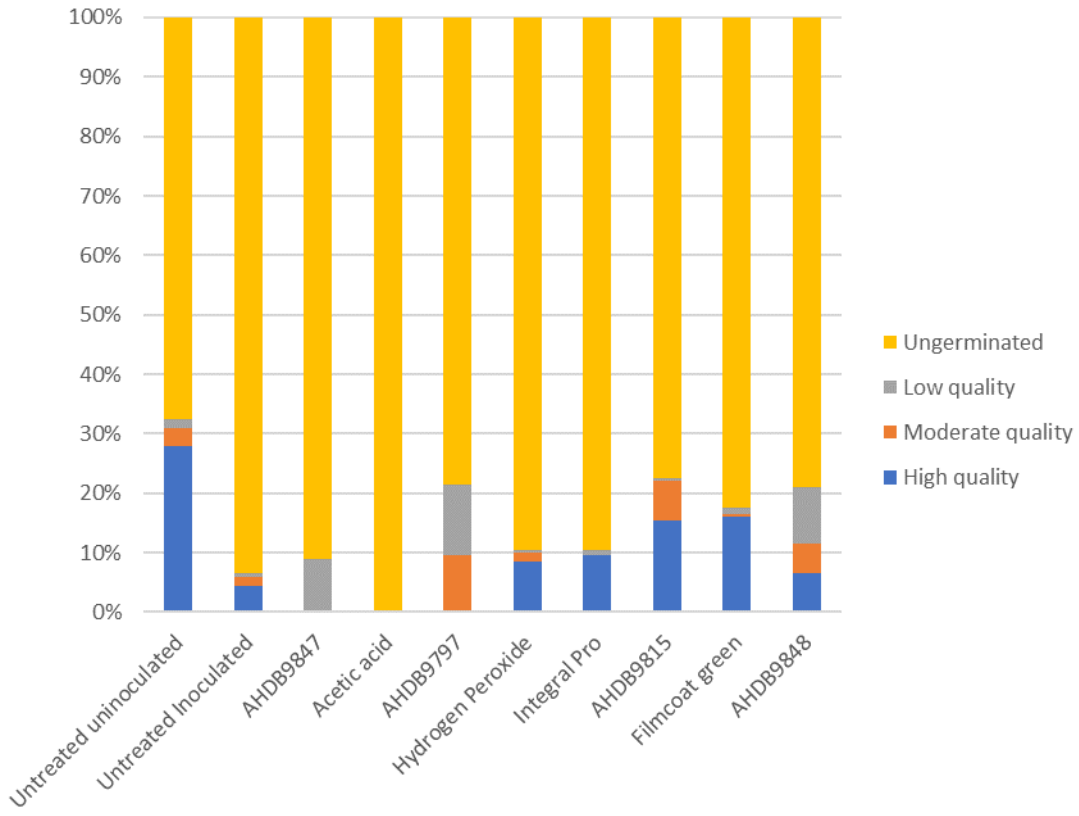


Figure 2. Distribution of quality categories for tested seeds (200 per treatment). Ungerminated is ungerminated viable and ungerminated unviable combined.

Table 6. Percentage of seeds in each quality category. Averages are based on trays with 50 seeds from 4 replicates. Data reported is from raw assessments but statistical analysis was performed on transformed data.

Treatment	Plant quality category (%)				
	High quality	**Moderate quality**	**Poor quality**	**Viable but ungerminated**	**Unviable and ungerminated**
Untreated uninoculated control	28	3	1.5	28.5	39
Untreated inoculated control	4.5	1.5	0.5	30.5	63
Filmcoat green	16	0.5	1	26.5	56
Acetic acid	0	0	0	0	100
AHDB9797	0	9.5	12	46.5	32
Hydrogen peroxide	8.5	1.5	0.5	24	65.5
Integral Pro	9.5	0	1	43.5	46
AHDB9815	15.5	6.5	0.5	41	36.5
AHDB9847	0	0	9	74	17
AHDB9848	6.5	5	9.5	23	56
p-value	<.001	0.575	0.004	<.001	<.001
d.f.	30	30	30	30	30
L.S.D.	5.35	5.097	3.564	9.88	9.75
Significantly different from the untreated control (P<0.05)					
Not significantly different from the untreated control					

Fresh weight

At the final assessment the untreated uninoculated and untreated inoculated controls had very similar average fresh weights (0.46g and 0.44g respectively) (Figure 3). AHDB9815 had the highest average fresh weight (0.92g), which was significantly different from other treatments and significantly higher than the untreated uninoculated control. The Filmcoat green treated seed was higher (0.57g) than both the untreated inoculated and uninoculated controls but not significantly different. All other treatments trended towards reduced fresh weight compared to the control but were not significantly different. AHDB9847 (0.08g) and acetic acid (0g) had the lowest average fresh weights (Figure 3, Table 7).

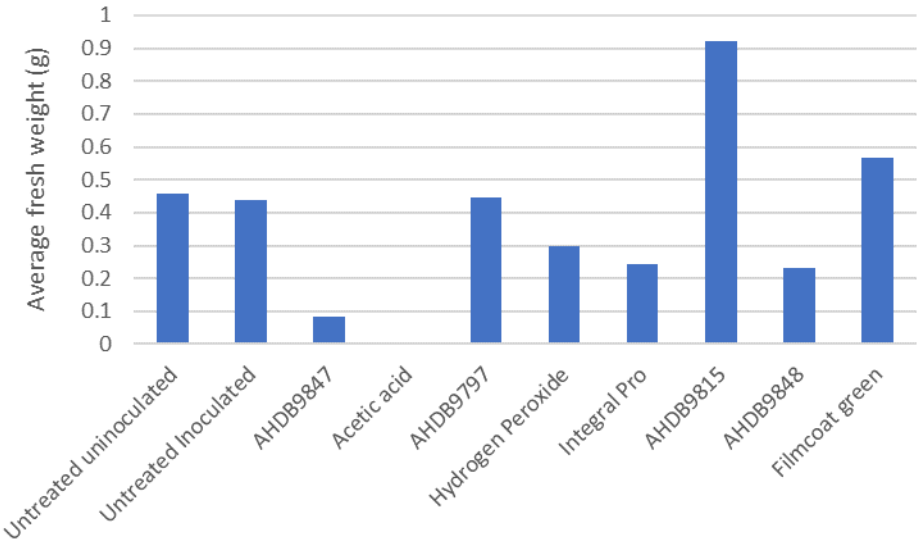


Figure 3. Average fresh weight (g) of fully emerged plants of combined plots for 10 treatments at the destructive assessment date 4 weeks after setting up the germination test.

Table 7. The average weight of all the fully emerged plants from each treatment in grams. Data reported is from raw assessments but statistical analysis was performed on transformed data.

Treatment	Mean weight of fully emerged plants (g)
Untreated uninoculated control	0.46
Untreated inoculated control	0.44
Filmcoat green	0.57
Acetic acid	0
AHDB9797	0.45
Hydrogen peroxide	0.30
Integral Pro	0.24
AHDB9815	0.92
AHDB9847	0.08
AHDB9848	0.23
p-value	0.003
d.f.	30
L.S.D.	0.3937
Significantly different from the untreated control P<0.05)	
Not significantly different from the untreated control	

Phytotoxicity

As expected phytotoxicity scores for the untreated inoculated and uninoculated controls were 0% (Figure 4). Hydrogen peroxide, Integral Pro, AHDB9815 and filmcoat green treated seeds also displayed no phytotoxicity. No seeds treated with acetic acid emerged and therefore were scored as 100% phytotoxicity. For those three treatments that did appear to cause some phytotoxicity this increased over time with AHDB9847 reaching 50% at the last assessment, AHDB9797 (30%) and AHDB9848 (20%). Symptoms observed were stunted thick roots, collapsed plants with curled growth, root browning and poor or no lateral root production.

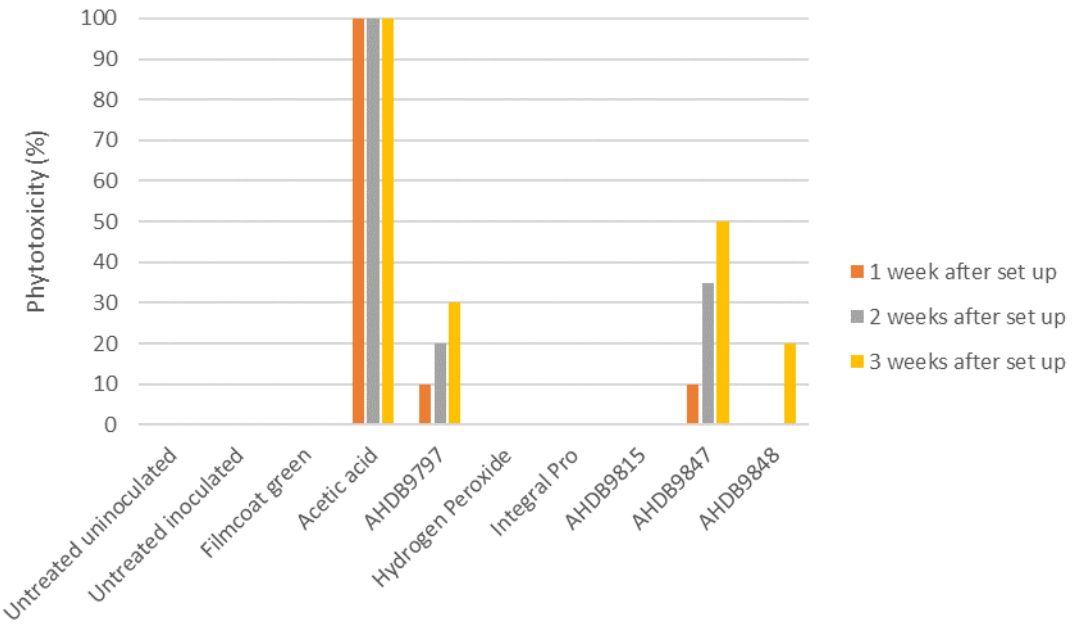


Figure 4. Mean percentage seedlings with symptoms of phytotoxicity assessed for 3 weeks after of set up of germinated test.

3. Efficacy of treatment to control pathogen growth from seed

Fungal growth on seeds

All treated and untreated spinach seeds developed some fungal growth by the final assessment at 7 days post plating (Figure 5), with the exception of acetic acid where only 7.3% (4 of 55 seeds) developed fungal growth. The majority of treatments developed fungal growth quickly with almost all seeds showing some fungal growth by 3 days. AHDB9797 and hydrogen peroxide had slightly slower growth, but by day 7 all seeds had visible mycelial growth.

Stemphylium was found in all treated seeds apart from those treated with acetic acid (Table 8). Identification of fungal colonies at 7 days showed that the untreated uninoculated control had a low level of Stemphylium present (3.6%). With the exception of the acetic acid and filmcoat green treatments, *Stemphylium* growth was significantly higher in treated seeds compared to the untreated uninoculated. The greatest number of seeds with confirmed Stemphylium present was in the untreated inoculated control (94.6% of the 55 tested), AHDB9847 (98.2%), AHDB9848 (92.7%) and AHDB9797 (92.7%). Acetic acid had no Stemphylium present and filmcoat green had a low level (12.7%). Filmcoat green had more Stemphylium than the uninoculated untreated control but it was significantly less than all of the other treatments.

Other colonies were identified as *Penicillium* spp., *Fusarium* spp., *Pythium* spp., *Botrytis* spp. and some other unidentified cultures and yeasts. *Penicillium* was the most common with the

other groups relatively few. The filmcoat green only treatment had 83.6% of seeds with *Penicillium* growth and AHDB9815 also had 50.9% of seeds.

There were more unknown/unidentified fungal colonies in the untreated uninoculated seeds than in any other treatment (80%).

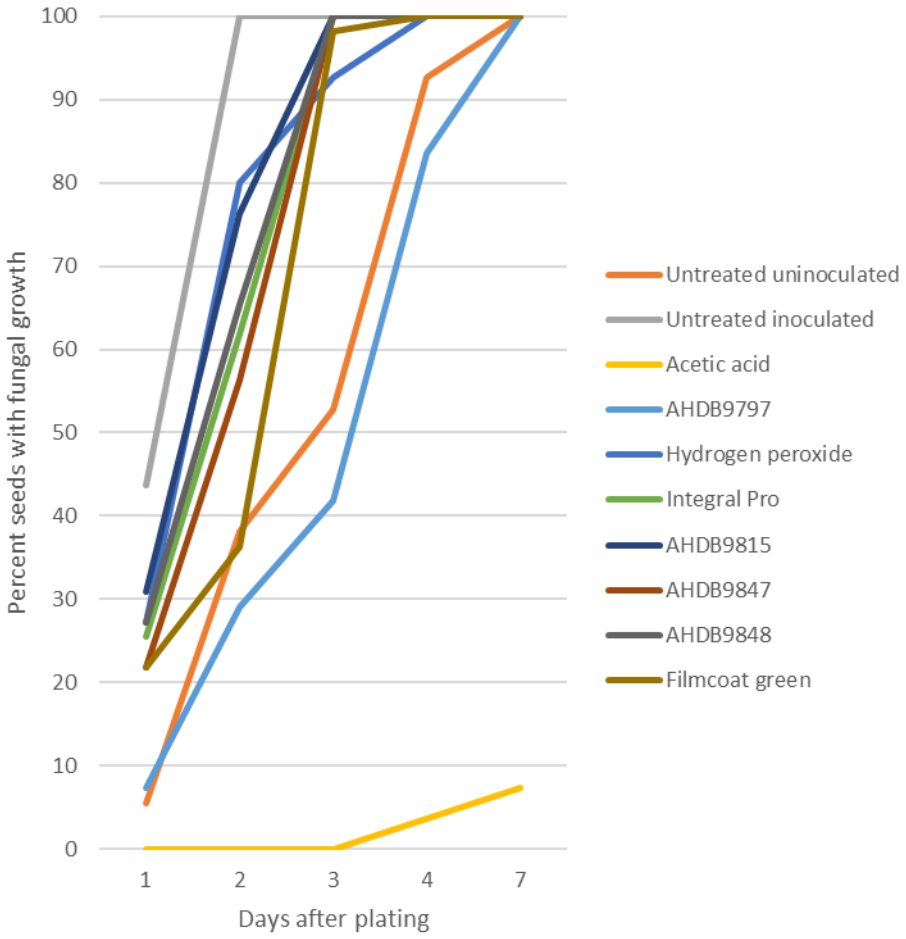


Figure 5. Percentage of seeds with fungal growth after plating onto agar. Assessments were done on 55 seeds per treatment with plots of five seeds per plate.

Table 8. Fungal growth on seeds at final assessment (7 days after plating). Percentage of seeds identified to have each fungal genus growing from them in the agar plating test. Eleven plates with 5 seeds each (total 55 seeds) for each treatment. Data reported is from raw assessments but statistical analysis was performed on transformed data.

Treatment	Fungal growths on seeds							
	Stemphylium	Penicillium	Fusarium	Pythium	Botrytis	Unknown	Yeast	No growth
Untreated uninoculated control	3.64	7.27	0.00	0.00	3.64	80.00	5.45	0.00
Untreated inoculated control	94.55	5.45	0.00	0.00	0.00	0.00	0.00	0.00
Filmcoat green	12.73	83.64	3.64	0.00	0.00	0.00	0.00	0.00
Acetic acid	0.00	5.45	0.00	0.00	0.00	1.82	0.00	92.73
AHDB9797	92.73	7.27	0.00	0.00	0.00	0.00	0.00	0.00
Hydrogen peroxide	63.64	21.82	0.00	12.73	0.00	1.82	0.00	0.00
Integral Pro	61.82	32.73	1.82	0.00	0.00	0.00	0.00	3.64
AHDB9815	50.91	49.09	0.00	0.00	0.00	0.00	0.00	0.00
AHDB9847	98.18	1.82	0.00	0.00	0.00	0.00	0.00	0.00
AHDB9848	92.73	3.64	0.00	0.00	0.00	1.82	0.00	1.82
p-value	<.001	<.001						
d.f.	100	100						
L.S.D.	12.26	12.08						
Significantly different from the untreated control								
Not significantly different from the untreated control								

Discussion:

Three chemical treatments, two conventional fungicides and two biological products were trialed in this study. As Thiram, the previous industry standard, is now unavailable it was not possible to include this as a standard for comparison.

S. botryosum is a seedborne pathogen that survives on plant debris is not generally thought to cause damping off in seedlings. The disease generally expresses as leaf spots on developing leaves. Inoculating the seed prior to treatment is a good model for the seedborne route of transmission for the pathogen. This study required substantial preliminary work to determine the most effective method to use. In particular the efficacy testing required a method that would successfully express the disease phenotype to allow an assessment of Stemphylium control.

Germination tests were conducted on inoculated treated seeds. Ideally these germination tests would have been conducted on both inoculated and uninoculated treated seeds for comparison but time and budget constraints meant that treatments could only be applied to inoculated seeds.

Germination tests showed that the baseline average percentage germination of the uninoculated untreated seeds was low (34.5%). Uninoculated seeds would be expected to have a higher germination rate, closer to 100% in a commercial setting. This poor baseline germination success rate could be due to age of the seed batch, which may lost some viability over time during the preliminary work on this project. The untreated inoculated seeds served as a baseline for impact of disease in absence of seed treatment and germination was very low (8%) indicating that the inoculation was successful and that the pathogen has a strong impact on germination rate in the absence of treatments.

The application of treatments and inoculation heavily impacted germination, but the differences between treatments do provide some information on the relative impact of the treatments on the seed and their respective control of Stemphylium. Treated seeds had generally lower germination rates between that of the untreated uninoculated and the untreated inoculated controls. This indicates that while treatments had some impact on germination there was also some efficacy against disease. Germination rates for filmcoat green, AHDB9797, AHDB9815, AHDB9848 were not significantly different from the untreated uninoculated control indicating that they had both some control of both disease and did not adversely effect the seeds.

To test efficacy of seed treatments specifically against Stemphylium a number of methods were trialed. Initially spinach seeds were inoculated with Stemphylium and grown on to larger plants to observe if leaf spot symptoms would develop sufficiently. This method did not successfully induce disease symptom developments on leaves, despite the seeds used being inoculated. The rate of seed inoculation was relatively high, so it may be that the growing conditions (i.e. limited air flow and agitation that would be present in a natural growing environment were not conducive to leaf spot development, rather than insufficient inoculum present.

Freeze blotter assays were also trialed in preliminary work. These assays are often used to determine the presence of a pathogen on seed. Freezing the seed prevents the seed from germinating and allows only those resident fungal and bacterial species present to grow. In preliminary trials fungal structures did grow from seeds successfully. However, it was not possible to observe the spore structures of Stemphylium on the seeds to identify the growth. The agar plating method was developed as an alternative to that would allow identification of fungi growing on and from seeds more easily. Seeds usually require 1 week to germinate but in these agar trials the pathogen grew out quickly, within 7 days and it was possible to identify Stemphylium from the growing cultures. The untreated uninoculated control had Stemphylium growth indicating some low level natural infection in this seed batch. Seeds with other treatments applied had much higher percentages of Stemphylium growth. This would indicate that the treatments were ineffective in controlling Stemphylium. The inoculum concentration used was designed to be realistic but treatments could have been more effective at lower inoculum rates and greater differences between treatments might be seen.

Chemical treatments, at the rates used, did not perform well in comparison to the other treatments tested. The Acetic acid rate was damaging, but lower rates could be trialed in the future to find a rate that balances fungal inhibition with plant development. Hydrogen peroxide was very similar to the untreated inoculated control indicating that it was not effective. AHDB9797 is very alkaline which makes the germination and growing environment very challenging, and there was poor efficacy.

The Filmcoat green treatment was the only treatment in the agar tests that reduced the incidence of Stemphylium to a level comparable with the untreated uninoculated control, without damaging the seeds. However, the filmcoat green treated seeds had the highest incidence of Penicillium. In the absence of a treatment that could control the Penicillium it could be that it was able to outcompete the Stemphylium for space and resources and

therefore masked the presence of Stemphylium. For the treated seeds the treatment may have been effective in preventing Penicillium growth but not effective against Stemphylium, which then allowed the Stemphylium to grow out and be identified on the agar. Penicillium might negatively impact germination rates so one might expect the filmcoat green germination rate to be one of the worst, but it was in the middle of the pack in this study.

Biological products AHDB9815 and Integral Pro did not appear to cause any phytotoxicity. AHDB9815 was overall the best performer of all treatments in all categories. It had a greater number of high-quality plants compared with Integral Pro and plant fresh weight was greater than the untreated uninoculated control suggesting a possible vigour advantage. AHDB9815 treated seeds developed Stemphylium infection in the agar seed plating experiment but the percentage of seeds with growth was lower than other treatments. Interestingly all those seeds treated with AHDB9815 that did not have Stemphylium growth, had Penicillium growth present instead (approx. 50:50). This result could be interpreted that AHDB9815 is better than other treatments at controlling Stemphylium indicated by the lower Stemphylium percentage recorded. However, an alternative interpretation could be that this treatment was not as good at controlling Penicillium as other treatments, which allowed the Penicillium to outcompete the Stemphylium on a higher percentage of seeds, rather than effectively controlling the Stemphylium. Penicillium present on seeds would also impact negatively impact germination.

It is possible that the Penicillium was introduced during the application of the filmcoat green as it would be expected to see similarly high levels of Penicillium on the untreated uninoculated control if the Penicillium was present as a natural infection, but this was not observed.

Conclusions:

Due to overall low germination rates of the seed batch, it is difficult to be fully confident in making conclusions about treatment effects.

Overall the best performing product was AHDB9815, a biological treatment, which resulted in the highest germination rate, plant quality and fresh weight, and no observed phytotoxicity. In the agar plating efficacy test there was Stemphylium growth less than in the other treatments. The inoculum rate used may have been too high and have overwhelmed treatments so that none gave very high levels of control, despite relative differences between treatments. The other biological product Integral Pro, had poor germination rate, but no phytotoxicity and a high proportion of seeds with Stemphylium growth.

The chemical treatments did not perform well at the rates used. Acetic acid, at the rate used, was too harsh a treatment. Although it inhibited all Stemphylium growth it also prevented germination completely. Hydrogen peroxide had poor germination rates, low quality plants and high number of seeds with Stemphylium growth.

AHDB9797 had relatively good germination but poor plant quality, signs of phytotoxicity and Stemphylium detected in numbers comparable to the untreated inoculated control.

AHDB9848 was the better performer of the two conventional fungicides. It had one of the highest germination rates but showed phytotoxic effects and had a high proportion of seeds with Stemphylium detected. The other conventional fungicide AHDB 9847 was one of the worst performers across all metrics measured and does not appear to offer good protection against *S. botryosum*.

Filmcoat green treated seeds had reduced Stemphylium growth, but this effect is likely to be due to the inability to control Penicillium and it outcompeting the Stemphylium and masking its presence.

Acknowledgements

AHDB for funding the work, and the crop protection companies for their financial contributions and provision of samples for the trials. Thanks to G's Fresh for providing Spinach seeds for this project work to be conducted.

Appendix

Classification of plant	Description of classification
High quality plants	Germinated with normal development: Cotyledons at least 50% emerged with no damage to terminal bud, roots over 1.0 cm.
Moderate quality of plants	Germinated with weak growth, and roots 0.5 – 1.0 cm.
Low quality of plants	Germinated with abnormal growth (such as twisting, necrotic spots), and roots less than 0.5 cm.
Viable ungerminated seeds	Ungerminated viable seed: Seeds which remain firm and apparently viable at the end of the test.
Unviable ungerminated seeds	Ungerminated dead seed: Seeds which at the end of the test period were either decayed, mouldy or soft.

a. Crop diary – events related to growing crop

Crop	Cultivar	Treatment date
Spinach	Monterey	October 2021

b. Table showing sequence of events by date – this relates to treatments and assessments.

Date	Event
	Efficacy tests
16.11.21	Assessment 1: disease incidence
17.11.21	Assessment 2: disease incidence
18.11.21	Assessment 3: disease incidence
19.11.21	Assessment 4: disease incidence
22.11.21	Assessment 5: disease incidence and ID
Germination tests representing commercial storage – ADAS	
11.11.21	Germination Assessment1
18.11.21	Germination assessment 2
25.11.21	Germination assessment 3
02.12.21	Germination assessment 4 Plant quality and weight

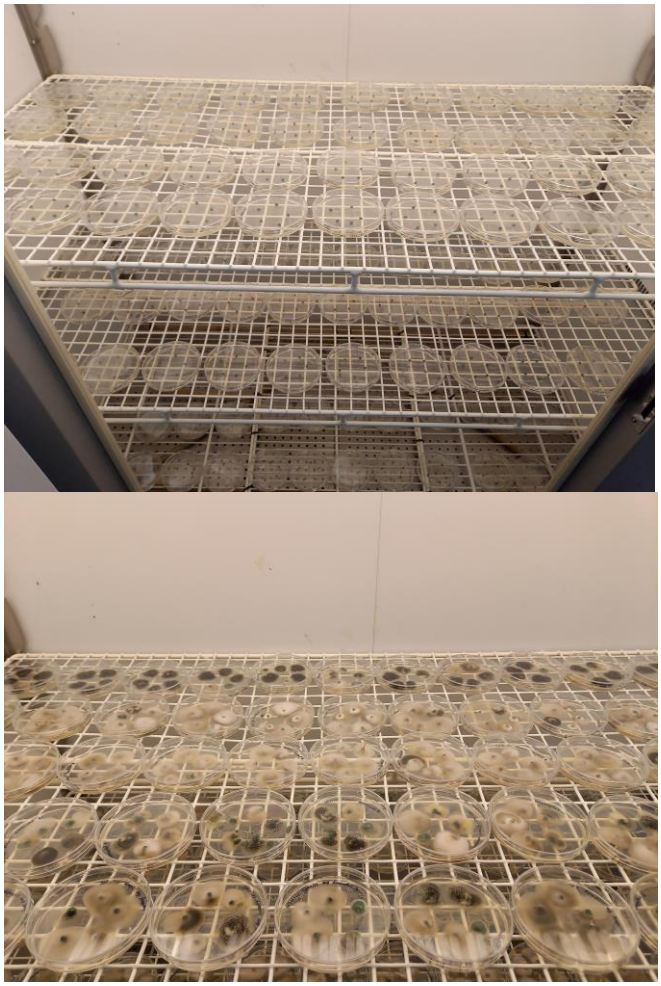
Growth stage:

Figure 1. The BBCH Growth stages from germination to harvest.

Growth stage	Code	Description
0: Germination	00	Dry seed
	01	Beginning of seed imbibition
	03	Seed imbibition complete
	05	Radicle emerged from seed
	07	Hypocotyl with cotyledons breaking through seed coat
	09	Emergence: cotyledons break through soil surface
1: Leaf development (Main shoot)	10	Cotyledons completely unfolded; growing point or true leaf initial visible
	11	First true leaf unfolded
	12	2nd true leaf unfolded
	13	3rd true leaf unfolded
	1 .	Stages continuous till . . .
	19	9 or more true leaves unfolded
3: Stem elongation of rosette growth	33	Leaf rosette has reached 30% of the expected diameter typical for the variety.[1] Main shoot has reached 30% of the expected height typical for the variety[2]
	35	Leaf rosette has reached 50% of the expected diameter typical for the variety.[1] Main shoot has reached 50% of the expected height typical for the variety[2]
	37	Leaf rosette has reached 70% of the expected diameter typical for the variety.[1] Main shoot has reached 70% of the expected height for the variety[2]
	39	Rosette development completed[1] Main shoot has reached the height typical for the variety[2]
4: Development of harvestable vegetative plant parts	41	10% of the leaf mass typical for the variety reached
	42	20% of the leaf mass typical for the variety reached
	43	30% of the leaf mass typical for the variety reached
	44	40% of the leaf mass typical for the variety reached
	45	50% of the leaf mass typical for the variety reached
	46	60% of the leaf mass typical for the variety reached
	47	70% of the leaf mass typical for the variety reached
	48	80% of the leaf mass typical for the variety reached
	49	Typical leaf mass reached

Photos:

Figure 2. Plot photo showing the Disease development experiment after set up (Top) and Disease development experiment at the end (Middle) and an example of treatment of **AHDB9847**, with *S. botryosum* growing from the seeds (Bottom).



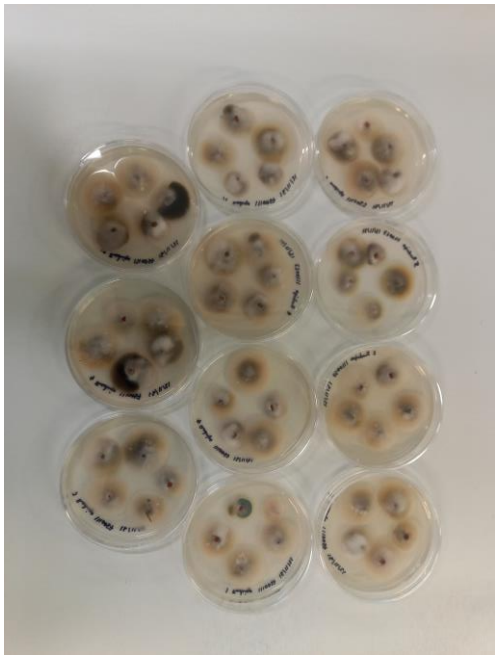


Figure 3. Plot photo showing the Disease development experiment with four different examples of *S. botryosum* that were identified under the microscope.

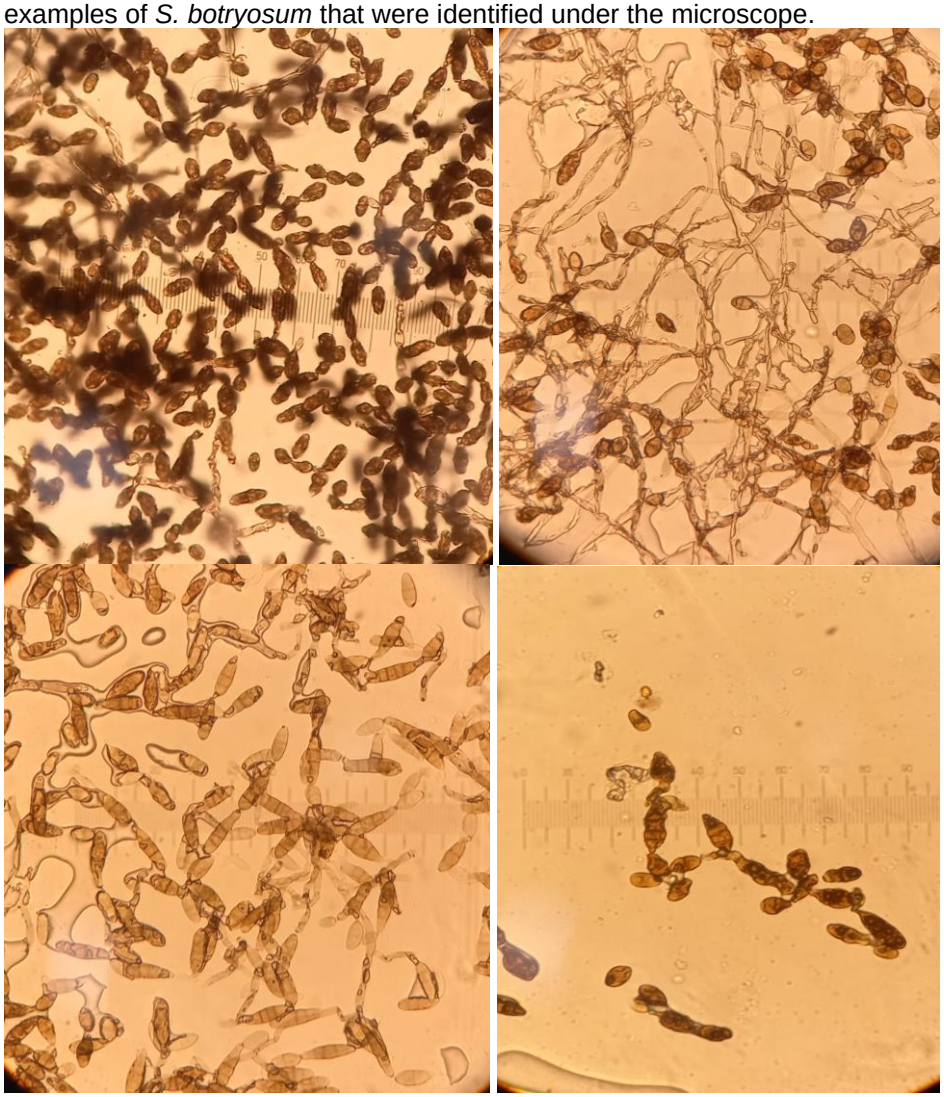
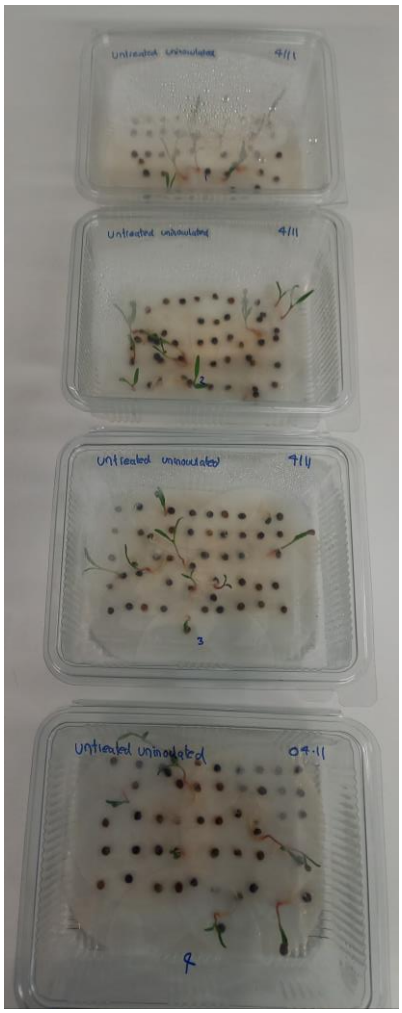
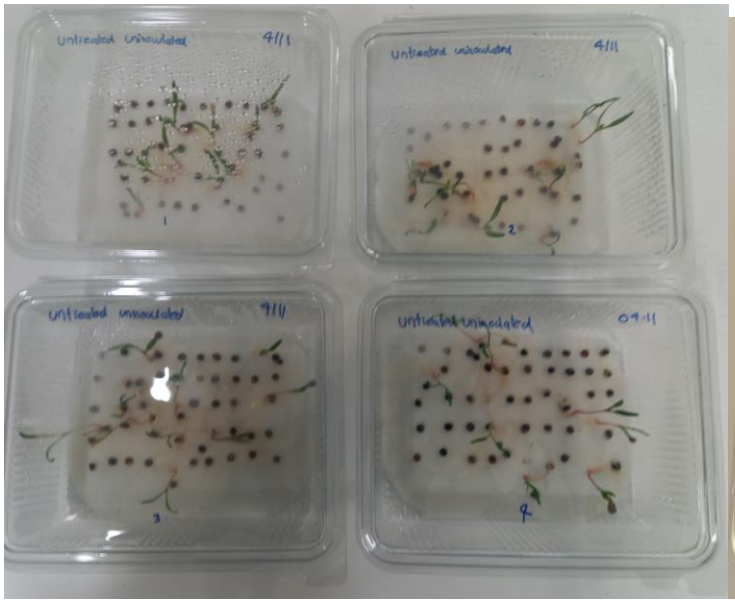
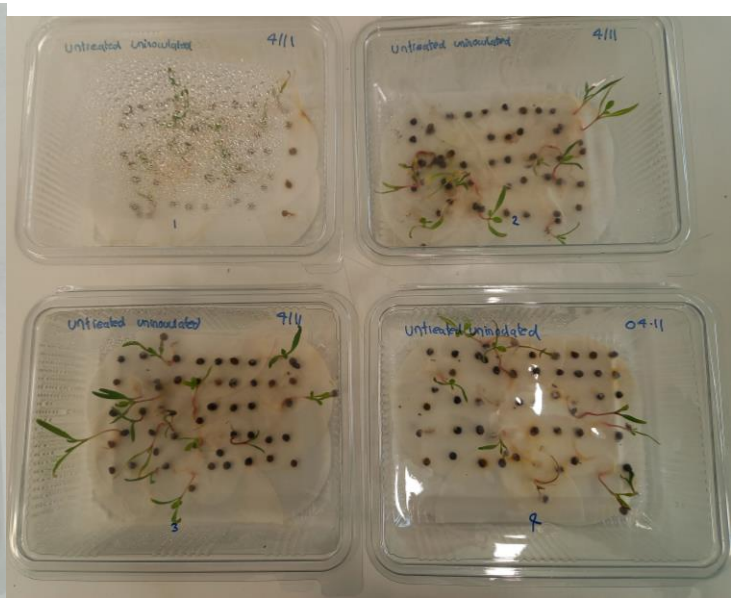


Figure 4. Three images of the untreated uninoculated control as it grows across the course of three weeks.




Untreated uninoculated 4/11
1
Untreated uninoculated 4/11
2
Untreated uninoculated 4/11
3
Untreated uninoculated 04.11
4


Untreated uninoculated 4/11
1
Untreated uninoculated 4/11
2
Untreated uninoculated 4/11
3
Untreated uninoculated 04.11
4

Raw data from assessments:

Figure 5. Plant quality assessment Raw data

Treatment code	Tray_number	High_qality_ plants	moderate_quality_ plants	Poor_quality_ plants	Viable_ungerm inated	Unviable_un germinated
Untreated uninoculated	**1**	21	5	0	11	13
Untreated uninoculated	**2**	14	0	0	17	19
Untreated uninoculated	**3**	14	1	0	12	23
Untreated uninoculated	**4**	7	0	3	17	23
Untreated Inoculated	**1**	2	2	0	8	38
Untreated Inoculated	**2**	2	1	0	20	27
Untreated Inoculated	**3**	3	0	0	13	34
Untreated Inoculated	**4**	2	0	1	20	27
AHDB9847	**1**	0	0	6	38	6
AHDB9847	**2**	0	0	3	38	9
AHDB9847	**3**	0	0	6	36	8
AHDB9847	**4**	0	0	3	36	11
Acetic acid	**1**	0	0	0	0	50
Acetic acid	**2**	0	0	0	0	50
Acetic acid	**3**	0	0	0	0	50
Acetic acid	**4**	0	0	0	0	50
AHDB9797	**1**	0	0	9	30	11
AHDB9797	**2**	0	0	5	22	23
AHDB9797	**3**	0	19	0	20	11
AHDB9797	**4**	0	0	10	21	19
Hydrogen Peroxide	**1**	11	2	1	8	28
Hydrogen Peroxide	**2**	3	0	0	10	37
Hydrogen Peroxide	**3**	0	1	0	16	33
Hydrogen Peroxide	**4**	3	0	0	14	33
Integral Pro	**1**	2	0	0	48	0
Integral Pro	**2**	0	0	1	17	32
Integral Pro	**3**	5	0	0	15	30
Integral Pro	**4**	12	0	1	7	30
AHDB9815	**1**	6	5	0	18	21
AHDB9815	**2**	11	3	0	25	11
AHDB9815	**3**	10	3	1	16	20
AHDB9815	**4**	4	2	0	23	21
Filmcoat green	**1**	16	1	2	5	26
Filmcoat green	**2**	2	0	0	17	31
Filmcoat green	**3**	6	0	0	12	32
Filmcoat green	**4**	8	0	0	19	23
AHDB9848	**1**	0	10	13	11	16
AHDB9848	**2**	2	0	4	11	33
AHDB9848	**3**	4	0	2	9	35
AHDB9848	**4**	7	0	0	15	28

Figure 6. Plant weight assessment Raw data

Treatment code	Tray_number	Weight_of_full_emerged_plants_(g)
Untreated uninoculated	**1**	0.62
Untreated uninoculated	**2**	0.45
Untreated uninoculated	**3**	0.54
Untreated uninoculated	**4**	0.22
Untreated Inoculated	**1**	0.45
Untreated Inoculated	**2**	0.29
Untreated Inoculated	**3**	0.53
Untreated Inoculated	**4**	0.49
AHDB9847	**1**	0.14
AHDB9847	**2**	0.05
AHDB9847	**3**	0.08
AHDB9847	**4**	0.06
Acetic acid	**1**	0
Acetic acid	**2**	0
Acetic acid	**3**	0
Acetic acid	**4**	0
AHDB9797	**1**	0.18
AHDB9797	**2**	0.2
AHDB9797	**3**	0.91
AHDB9797	**4**	0.5
Hydrogen Peroxide	**1**	0.91
Hydrogen Peroxide	**2**	0.16
Hydrogen Peroxide	**3**	0.01
Hydrogen Peroxide	**4**	0.12
Integral Pro	**1**	0.15
Integral Pro	**2**	0.08
Integral Pro	**3**	0.26
Integral Pro	**4**	0.48
AHDB9815	**1**	1.05
AHDB9815	**2**	1.31
AHDB9815	**3**	0.84
AHDB9815	**4**	0.48
Filmcoat green	**1**	1.21
Filmcoat green	**2**	0.29
Filmcoat green	**3**	0.43
Filmcoat green	**4**	0.34
AHDB9848	**1**	0.64
AHDB9848	**2**	0.1
AHDB9848	**3**	0.07
AHDB9848	**4**	0.12

Figure 7. Seed germination rate over 3 weeks Raw data

Treatment code	Tray_number	Assessment_1_11.11.21	Assessment_2_18.11.21	Assessment_3_25.11.21
Untreated uninoculated	1	10	10	29
Untreated uninoculated	2	11	11	13
Untreated uninoculated	3	9	11	15
Untreated uninoculated	4	11	11	12
Untreated Inoculated	1	2	4	6
Untreated Inoculated	2	2	4	4
Untreated Inoculated	3	2	3	3
Untreated Inoculated	4	3	3	3
Filmcoat Green	1	14	16	19
Filmcoat Green	2	4	4	4
Filmcoat Green	3	6	6	6
Filmcoat Green	4	5	8	9
Acetic acid	1	0	0	0
Acetic acid	2	0	0	0
Acetic acid	3	0	0	0
Acetic acid	4	0	0	0
AHDB9797	1	5	8	9
AHDB9797	2	4	6	6
AHDB9797	3	15	17	18
AHDB9797	4	11	11	11
Hydrogen Peroxide	1	1	5	13
Hydrogen Peroxide	2	0	3	4
Hydrogen Peroxide	3	4	4	4
Hydrogen Peroxide	4	1	4	4
Integral Pro	1	2	2	2
Integral Pro	2	4	4	4
Integral Pro	3	4	5	5
Integral Pro	4	9	9	12
AHDB9815	1	5	5	13
AHDB9815	2	13	19	17
AHDB9815	3	8	10	19
AHDB9815	4	5	7	8
AHDB9847	1	8	8	8
AHDB9847	2	3	3	3
AHDB9847	3	2	5	5
AHDB9847	4	3	4	4
AHDB9848	1	11	23	37
AHDB9848	2	0	3	6
AHDB9848	3	2	4	11
AHDB9848	4	6	8	7

Figure 8. Plant Disease development assessment Raw data

Part 1 of 2

Treatment code	Plate_Number	Fungal_Growth_on_Seed_Assessment_1_16.11.21	Fungal_Growth_on_Seed_Assessment_2_17.11.21	Fungal_Growth_on_Seed_Assessment_3_18.11.21	Fungal_Growth_on_Seed_Assessment_4_19.11.21	Fungal_Growth_on_Seed_Assessment_5_22.11.21
Untreated uninoculated	**1**	1	3	3	5	5
Untreated uninoculated	**2**	0	1	3	5	5
Untreated uninoculated	**3**	0	1	2	5	5
Untreated uninoculated	**4**	1	2	3	4	5
Untreated uninoculated	**5**	1	3	4	5	5
Untreated uninoculated	**6**	0	0	0	4	5
Untreated uninoculated	**7**	0	2	2	4	5
Untreated uninoculated	**8**	0	2	3	5	5
Untreated uninoculated	**9**	0	2	4	5	5
Untreated uninoculated	**10**	0	2	2	5	5
Untreated uninoculated	**11**	0	3	3	4	5
Untreated Inoculated	**1**	2	5	5	5	5
Untreated Inoculated	**2**	2	5	5	5	5
Untreated Inoculated	**3**	1	5	5	5	5
Untreated Inoculated	**4**	3	5	5	5	5
Untreated Inoculated	**5**	2	5	5	5	5
Untreated Inoculated	**6**	1	5	5	5	5
Untreated Inoculated	**7**	3	5	5	5	5
Untreated Inoculated	**8**	1	5	5	5	5
Untreated Inoculated	**9**	3	5	5	5	5
Untreated Inoculated	**10**	2	5	5	5	5
Untreated Inoculated	**11**	4	5	5	5	5
Acetic acid	**1**	0	0	0	0	1
Acetic acid	**2**	0	0	0	0	0
Acetic acid	**3**	0	0	0	0	0
Acetic acid	**4**	0	0	0	0	0
Acetic acid	**5**	0	0	0	0	0
Acetic acid	**6**	0	0	0	1	1
Acetic acid	**7**	0	0	0	0	0
Acetic acid	**8**	0	0	0	1	1
Acetic acid	**9**	0	0	0	0	0
Acetic acid	**10**	0	0	0	0	1
Acetic acid	**11**	0	0	0	0	0
AHDB9797	**1**	0	1	2	3	5
AHDB9797	**2**	0	1	2	3	5
AHDB9797	**3**	0	0	1	5	5
AHDB9797	**4**	1	2	2	5	5
AHDB9797	**5**	0	0	0	3	5
AHDB9797	**6**	2	3	4	4	5
AHDB9797	**7**	0	4	4	5	5
AHDB9797	**8**	0	2	4	5	5
AHDB9797	**9**	0	1	1	4	5
AHDB9797	**10**	0	0	1	4	5
AHDB9797	**11**	1	2	2	5	5
Hydrogen Peroxide	**1**	0	3	5	5	5
Hydrogen Peroxide	**2**	0	3	5	5	5
Hydrogen Peroxide	**3**	2	4	5	5	5
Hydrogen Peroxide	**4**	0	3	5	5	5
Hydrogen Peroxide	**5**	2	4	5	5	5
Hydrogen Peroxide	**6**	1	4	5	5	5
Hydrogen Peroxide	**7**	1	4	4	5	5
Hydrogen Peroxide	**8**	2	5	3	5	5
Hydrogen Peroxide	**9**	2	4	5	5	5
Hydrogen Peroxide	**10**	2	5	4	5	5
Hydrogen Peroxide	**11**	3	5	5	5	5

Part 2 of 2						
Treatment code	Plate_Number	Fungal_Growth_on_Seed_Assessment_1_16.11.21	Fungal_Growth_on_Seed_Assessment_2_17.11.21	Fungal_Growth_on_Seed_Assessment_3_18.11.21	Fungal_Growth_on_Seed_Assessment_4_19.11.21	Fungal_Growth_on_Seed_Assessment_5_22.11.21
Integral Pro	1	0	2	5	5	5
Integral Pro	2	0	3	5	5	5
Integral Pro	3	2	2	5	5	5
Integral Pro	4	1	2	5	5	5
Integral Pro	5	2	4	5	5	5
Integral Pro	6	1	3	5	5	5
Integral Pro	7	2	5	5	5	5
Integral Pro	8	3	4	5	5	5
Integral Pro	9	0	2	5	5	5
Integral Pro	10	2	3	5	5	5
Integral Pro	11	1	4	5	5	5
AHDB9815	1	1	5	5	5	5
AHDB9815	2	0	3	5	5	5
AHDB9815	3	1	2	5	5	5
AHDB9815	4	0	2	5	5	5
AHDB9815	5	2	4	5	5	5
AHDB9815	6	4	5	5	5	5
AHDB9815	7	1	3	5	5	5
AHDB9815	8	1	4	5	5	5
AHDB9815	9	2	4	5	5	5
AHDB9815	10	3	5	5	5	5
AHDB9815	11	2	5	5	5	5
AHDB9847	1	2	2	5	5	5
AHDB9847	2	1	3	5	5	5
AHDB9847	3	1	3	5	5	5
AHDB9847	4	0	2	5	5	5
AHDB9847	5	1	4	5	5	5
AHDB9847	6	0	0	5	5	5
AHDB9847	7	2	4	5	5	5
AHDB9847	8	3	5	5	5	5
AHDB9847	9	2	4	5	5	5
AHDB9847	10	0	1	5	5	5
AHDB9847	11	0	3	5	5	5
AHDB9848	1	3	4	5	5	5
AHDB9848	2	1	3	5	5	5
AHDB9848	3	0	3	5	5	5
AHDB9848	4	0	3	5	5	5
AHDB9848	5	0	2	5	5	5
AHDB9848	6	2	4	5	5	5
AHDB9848	7	1	3	5	5	5
AHDB9848	8	4	4	5	5	5
AHDB9848	9	3	4	5	5	5
AHDB9848	10	0	2	5	5	5
AHDB9848	11	1	4	5	5	5
Filmcoat green	1	2	2	5	5	5
Filmcoat green	2	1	2	5	5	5
Filmcoat green	3	3	3	5	5	5
Filmcoat green	4	2	3	5	5	5
Filmcoat green	5	1	1	4	5	5
Filmcoat green	6	1	2	5	5	5
Filmcoat green	7	1	1	5	5	5
Filmcoat green	8	0	0	5	5	5
Filmcoat green	9	0	3	5	5	5
Filmcoat green	10	0	1	5	5	5
Filmcoat green	11	1	2	5	5	5

Figure 9. Plant Disease identification assessment Raw data

Treatment code	Plate_Number	Stemphylium_count	Penacilium_count	Fusarium_count	Pythium_count	Botrytis	Unknown	Yeast	Unaffected_Seed
Untreated uninoculated	1	1	1	0	0	0	3	0	0
Untreated uninoculated	2	0	1	0	0	0	4	0	0
Untreated uninoculated	3	0	0	0	0	2	3	0	0
Untreated uninoculated	4	0	1	0	0	0	3	1	0
Untreated uninoculated	5	0	0	0	0	0	5	0	0
Untreated uninoculated	6	0	1	0	0	0	3	1	0
Untreated uninoculated	7	0	0	0	0	0	4	1	0
Untreated uninoculated	8	1	0	0	0	0	4	0	0
Untreated uninoculated	9	0	0	0	0	0	5	0	0
Untreated uninoculated	10	0	0	0	0	0	5	0	0
Untreated uninoculated	11	0	0	0	0	0	5	0	0
Untreated Inoculated	1	5	0	0	0	0	0	0	0
Untreated Inoculated	2	5	0	0	0	0	0	0	0
Untreated Inoculated	3	5	0	0	0	0	0	0	0
Untreated Inoculated	4	3	2	0	0	0	0	0	0
Untreated Inoculated	5	5	0	0	0	0	0	0	0
Untreated Inoculated	6	5	0	0	0	0	0	0	0
Untreated Inoculated	7	5	0	0	0	0	0	0	0
Untreated Inoculated	8	5	0	0	0	0	0	0	0
Untreated Inoculated	9	5	0	0	0	0	0	0	0
Untreated Inoculated	10	4	1	0	0	0	0	0	0
Untreated Inoculated	11	5	0	0	0	0	0	0	0
Acetic acid	1	0	0	0	0	0	1	0	4
Acetic acid	2	0	0	0	0	0	0	0	5
Acetic acid	3	0	0	0	0	0	0	0	5
Acetic acid	4	0	0	0	0	0	0	0	5
Acetic acid	5	0	0	0	0	0	0	0	5
Acetic acid	6	0	1	0	0	0	0	0	4
Acetic acid	7	0	0	0	0	0	0	0	5
Acetic acid	8	0	1	0	0	0	0	0	4
Acetic acid	9	0	0	0	0	0	0	0	5
Acetic acid	10	0	1	0	0	0	0	0	4
Acetic acid	11	0	0	0	0	0	0	0	5
AHDB9797	1	4	1	0	0	0	0	0	0
AHDB9797	2	5	0	0	0	0	0	0	0
AHDB9797	3	3	2	0	0	0	0	0	0
AHDB9797	4	5	0	0	0	0	0	0	0
AHDB9797	5	4	1	0	0	0	0	0	0
AHDB9797	6	5	0	0	0	0	0	0	0
AHDB9797	7	5	0	0	0	0	0	0	0
AHDB9797	8	5	0	0	0	0	0	0	0
AHDB9797	9	5	0	0	0	0	0	0	0
AHDB9797	10	5	0	0	0	0	0	0	0
AHDB9797	11	5	0	0	0	0	0	0	0
Hydrogen Peroxide	1	4	1	0	0	0	0	0	0
Hydrogen Peroxide	2	4	1	0	0	0	0	0	0
Hydrogen Peroxide	3	4	1	0	0	0	0	0	0
Hydrogen Peroxide	4	2	1	0	2	0	0	0	0
Hydrogen Peroxide	5	3	0	0	2	0	0	0	0
Hydrogen Peroxide	6	5	0	0	0	0	0	0	0
Hydrogen Peroxide	7	1	2	0	2	0	0	0	0
Hydrogen Peroxide	8	1	3	0	0	0	1	0	0
Hydrogen Peroxide	9	3	1	0	1	0	0	0	0
Hydrogen Peroxide	10	4	1	0	0	0	0	0	0
Hydrogen Peroxide	11	4	1	0	0	0	0	0	0
Integral Pro	1	2	3	0	0	0	0	0	0
Integral Pro	2	3	2	0	0	0	0	0	0
Integral Pro	3	3	1	1	0	0	0	0	0
Integral Pro	4	1	4	0	0	0	0	0	0
Integral Pro	5	3	2	0	0	0	0	0	0
Integral Pro	6	4	1	0	0	0	0	0	0
Integral Pro	7	5	0	0	0	0	0	0	0
Integral Pro	8	2	2	0	0	0	0	0	1
Integral Pro	9	3	1	0	0	0	0	0	1
Integral Pro	10	4	1	0	0	0	0	0	0
Integral Pro	11	4	1	0	0	0	0	0	0
AHDB9815	1	3	2	0	0	0	0	0	0
AHDB9815	2	3	2	0	0	0	0	0	0
AHDB9815	3	2	3	0	0	0	0	0	0
AHDB9815	4	5	0	0	0	0	0	0	0
AHDB9815	5	3	2	0	0	0	0	0	0
AHDB9815	6	1	4	0	0	0	0	0	0
AHDB9815	7	2	3	0	0	0	0	0	0
AHDB9815	8	2	3	0	0	0	0	0	0
AHDB9815	9	1	4	0	0	0	0	0	0
AHDB9815	10	2	3	0	0	0	0	0	0
AHDB9815	11	4	1	0	0	0	0	0	0
AHDB9847	1	4	1	0	0	0	0	0	0
AHDB9847	2	5	0	0	0	0	0	0	0
AHDB9847	3	5	0	0	0	0	0	0	0
AHDB9847	4	5	0	0	0	0	0	0	0
AHDB9847	5	5	0	0	0	0	0	0	0
AHDB9847	6	5	0	0	0	0	0	0	0
AHDB9847	7	5	0	0	0	0	0	0	0
AHDB9847	8	5	0	0	0	0	0	0	0
AHDB9847	9	5	0	0	0	0	0	0	0
AHDB9847	10	5	0	0	0	0	0	0	0
AHDB9847	11	5	0	0	0	0	0	0	0
AHDB9848	1	2	1	0	0	0	1	0	1
AHDB9848	2	5	0	0	0	0	0	0	0
AHDB9848	3	5	0	0	0	0	0	0	0
AHDB9848	4	5	0	0	0	0	0	0	0
AHDB9848	5	5	0	0	0	0	0	0	0
AHDB9848	6	4	1	0	0	0	0	0	0
AHDB9848	7	5	0	0	0	0	0	0	0
AHDB9848	8	5	0	0	0	0	0	0	0
AHDB9848	9	5	0	0	0	0	0	0	0
AHDB9848	10	5	0	0	0	0	0	0	0
AHDB9848	11	5	0	0	0	0	0	0	0
Filmcoat green	1	2	3	0	0	0	0	0	0
Filmcoat green	2	0	5	0	0	0	0	0	0
Filmcoat green	3	0	5	0	0	0	0	0	0
Filmcoat green	4	1	4	0	0	0	0	0	0
Filmcoat green	5	2	3	0	0	0	0	0	0
Filmcoat green	6	1	3	1	0	0	0	0	0
Filmcoat green	7	0	5	0	0	0	0	0	0
Filmcoat green	8	0	5	0	0	0	0	0	0
Filmcoat green	9	1	4	0	0	0	0	0	0
Filmcoat green	10	0	5	0	0	0	0	0	0
Filmcoat green	11	0	4	1	0	0	0	0	0

Figure 10. Plant phytotoxicity assessment Raw data

Treatment code	Phytotoxicity_Assessment_1	Phytotoxicity_Assessment_2	Phytotoxicity_Assessment_3
Untreated uninoculated	0%	0%	0%
Untreated Inoculated	0%	0%	0%
Filmcoat green	0%	0%	0%
Acetic acid	100%	100%	100%
AHDB9797	10%	20%	30%
Hydrogen Peroxide	0%	0%	0%
Integral Pro	0%	0%	0%
AHDB9815	0%	0%	0%
AHDB9847	10%	35%	50%
AHDB9848	0%	0%	20%

Figure 11. A construction of the trial layout with plot order, block, treatment with top shelf shown in red and the bottom shelf shown in green. Filmcoat green, was an additional treatment and was not part of the randomization. This treatment was shelved separately.

Treatment			3	9	5	2	1	4	8	6	7	
Block			11	11	11	11	11	11	11	11	11	
Plot			1101	1102	1103	1104	1105	1106	1107	1108	1109	
Treatment			1	5	7	3	6	2	8	9	4	
Block			10	10	10	10	10	10	10	10	10	
Plot			1001	1002	1003	1004	1005	1006	1007	1008	1009	
Treatment			8	2	5	7	3	9	4	1	6	
Block			9	9	9	9	9	9	9	9	9	
Plot			901	902	903	904	905	906	907	908	909	Top
Treatment			6	4	1	8	9	2	3	7	5	
Block			8	8	8	8	8	8	8	8	8	
Plot			801	802	803	804	805	806	807	808	809	
Treatment			2	4	7	9	6	3	1	5	8	
Block			7	7	7	7	7	7	7	7	7	
Plot			701	702	703	704	705	706	707	708	709	
Treatment			3	5	2	1	4	9	6	7	8	
Block			6	6	6	6	6	6	6	6	6	
Plot			601	602	603	604	605	606	607	608	609	
Treatment			6	1	4	3	8	5	9	2	7	
Block			5	5	5	5	5	5	5	5	5	
Plot			501	502	503	504	505	506	507	508	509	Bottom
Treatment			3	1	2	4	8	9	6	5	7	
Block			4	4	4	4	4	4	4	4	4	
Plot			401	402	403	404	405	406	407	408	409	
Treatment			9	8	4	1	6	7	2	5	3	
Block			3	3	3	3	3	3	3	3	3	
Plot			301	302	303	304	305	306	307	308	309	
Treatment			6	9	3	4	2	7	8	1	5	
Block			2	2	2	2	2	2	2	2	2	
Plot			201	202	203	204	205	206	207	208	209	
Treatment			8	4	2	9	5	7	1	3	6	
Block			1	1	1	1	1	1	1	1	1	
Plot			101	102	103	104	105	106	107	108	109	

ORETO certificate.

Certificate of

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

This certifies that

RSK ADAS Ltd

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
Stored Crops
Biologicals and Semiochemicals

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

Signature *Authorised signatory*

Certification Number
ORETO 409

HSE
Chemicals Regulation Division

Department of Agriculture and Rural Development