SCEPTREPLUS

Final Trial Report

Trial code:	SP62a
Title:	Evaluation of new seed treatments for control of soil-borne Rhizoctonia in cauliflower
Crop	Field vegetables - cauliflower
Target	*Rhizoctonia solani*, RHIZSO
Lead researcher:	Dave Kaye (ADAS)
Organisation:	RSK ADAS Ltd.
Period:	Jan – June 2021
Report date:	30th June 2021
Report author:	Dave Kaye, Catherine Eyre
Report authorised by:	Angela Huckle
ORETO Number:	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: 02/07/2021 Authors signature: D. Kaye.

Grower Summary

Introduction

The fungal plant pathogen *Rhizoctonia solani* causes damping off in cauliflower. The disease is characterized by seed rotting, failed emergence or the death of seedlings soon after they emerge. Seedlings which do emerge develop a lesion at the stem base at the contact point with the soil and this leads to the collapse of the seedling. Some seedlings may manage to grow with the lesion, but in most instances they will eventually collapse.

Seed treatment is an important part of managing this disease. Until recently Agrichem Flowable Thiram (thiram) was the industry standard plant protection product used and was applied as a warm water soak. With the withdrawal of this product, alternative seed treatments are required to effectively manage the threat from *R. solani*. Non-chemical alternatives would benefit both conventional and organic growers, given the continued consumer and retailer pressure for a reduction in the use of chemical fungicide products. A trial conducted under controlled environmental conditions was designed to identify potential new seed treatments against *R. solani* for cauliflower.

Methods

Efficacy and crop safety experiments were conducted with cauliflower seed of the susceptible variety, Floriade. Seeds were subsampled and treated with 6 different seed treatments by Elsoms Seeds Ltd. As well as the treatment, all seeds received Filmcoat Green which is a standard commercial practice.

Phytotoxicity

The effect of the seed treatments on seed germination was assessed in two experiments, where seeds were sown onto moist filter paper in sealed plastic boxes.

1. 1 week post treatment – conducted by Elsoms
    - 100 seeds/treatment.
    - Scored for germination at 28 days.
2. 12 weeks post treatment – conducted by ADAS
    - 12 weeks represents medium-term commercial storage practices.
    - 100 seeds/treatment.
    - Scored for emergence at 7 and 14 days.
    - Scored for seedling quality (1-5 scale) at 14 days.

Seed treatment efficacy for control of *R. solani*

To test the disease control efficacy of the seed treatments they were challenged in a controlled experiment by sowing into growth medium inoculated with a pathogenic isolate of *R. solani*.

- Preliminary study was conducted to determine optimal *R. solani* inoculum concentration to use
- Main disease control efficacy trials
    - 4 replicates of 24 seeds.
    - Sown into 200 g of 10% inoculated growth medium, grown at 20˚C for 28 days.
    - Assessed at 5 time points up to 23 days.
    - Scored for germination (%) and vigour (%) at every time point.
    - Scored for disease incidence (%), disease severity (%) and fresh weight measured (g) at final assessment.

Results

Seed germination test

Germination 1 week post-treatment

- All treatments, except for AHDB9797, had very high germination rates (Table 1)
- Germination rate ranged between 95% (Untreated + Filmcoat Green, AHDB9849, AHDB9807, AHDB9850, AHDB9847) and 96% (AHDB9848).
- AHDB9797 had very poor germination rate (46%) indicating this treatment was phytotoxic.

Table 1. Germination of seeds 1 month after treatment, February 2021.

Treatment	Elsoms lot no.	Germination % final
Untreated	E71108	97
Untreated + Filmcoat green	E71158	95
AHDB9797	E71159	46
AHDB9849	E71160	95
AHDB9807	E71162	95
AHDB9850	E71164	95
AHDB9847	E71167	95
AHDB9848	E71168	96

Germination 12 weeks post-treatment

- All treatments, except AHDB9797, had high germination rates.
- 14 days after sowing 6 of 7 treatments were at 100% germination.
- AHDB9797 germination rate decreased compared with the 1 month post-treatment test from 46% to 28%.
- Seedling quality was good in all treatments apart from AHDB9797.
- AJDB9850 may cause a slight lag in development but had no longer term effects.

Table 2. Longer term storage of seeds (4 months). Germination and seedling quality at 7 and 14 days after sowing.

Treatment	Germination % 7 d	Seedling quality (1-5 index)	Germination % 14 d	Seedling quality (1-5 index)
Untreated	100	5	100	5
AHDB9797	8	2	28	3
AHDB9849	100	5	100	5
AHDB9807	100	5	100	5
AHDB9850	98	4	100	5
AHDB9847	97	5	100	5
AHDB9848	100	5	100	5

All seed was treated with Filmcoat Green

Seed treatment efficacy

Emergence

- Emergence of the untreated uninoculated control seed was 77% at the final assessment, 23 days after sowing.
- Untreated seed in *R. solani* inoculated soil had a low emergence rate as expected.
- All treatments, apart from AHDB9797 resulted in a higher emergence rate than the untreated inoculated control and AHDB9847 had the highest emergence rate of 84.6%.
- Although there was a trend of better performance with the treatments, results were not statistically significantly different from the untreated inoculated control (p=0.089).
- AHDB9797 resulted in the least control (25.7%) but poor performance was due to phytotoxicity rather than disease pressure.

Vigour

- Vigour for all treatments, apart from AHDB9797, was comparable to the untreated inoculated control but there were no significant differences between treatments (p=0.062).
- AHDB9849 resulted in the best vigour – exceeded the untreated inoculated control and matched the untreated uninoculated control.

Disease incidence and severity

- The highest disease incidence was in the untreated inoculated control treatment (25.9%) with 0% incidence in the uninoculated control (Table 3, Figure 2).
- AHDB9849, AHDB9850, AHDB9847 all appeared to control disease symptoms well and disease incidence and severity were significantly different from the untreated uninoculated control. AHDB9849 had the lowest disease incidence (0.3%).
- AHDB9797 and AHDB9807 had disease incidence comparable but not significantly different from the untreated inoculated control (19.8% and 11.9% respectively).

Fresh weight

- Seedling fresh weight was significantly different between treatments (p=0.003).
- AHDB9847 performed best – average sum of fresh weight 1.55g was significantly higher from the untreated uninoculated control.

Table 3. Effect of plant protection products on average germination, vigour, disease incidence, severity and the average sum of fresh weight of cauliflower seed at final assessment point 23 days after sowing. 4 replicates per treatment (4 x 24, n=96).

Treatment	Emerged %	DT	Vigour %	DT	Disease incidence %	DT	% control	Disease severity %	DT	% control	Fresh weight g	DT
Untreated uninoculated	76.7	b	100.0	b	0.00	a	100	0.00	a	100	1.95	d
Untreated inoculated	45.6	ab	89.0	ab	25.92	d	0	3.74	c	0	0.71	ab
AHDB9797	25.7	a	55.0	a	19.81	cd	23.6	2.26	bc	39.6	0.34	a
AHDB9849	72.8	b	100.0	b	0.26	ab	99.0	0.25	ab	93.3	1.05	abc
AHDB9807	54.6	ab	90.6	ab	11.89	bcd	54.1	4.00	c	-7.0	0.98	abc
AHDB9850	72.1	b	90.3	ab	0.54	ab	97.9	0.25	ab	93.3	1.36	bcd
AHDB9847	84.6	b	93.8	ab	2.00	ab	92.28	0.25	ab	93.3	1.55	cd
AHDB9848	73.5	b	97.9	b	3.63	abc	86.0	1.01	abc	73.0	1.07	abc
P value	0.089		0.062		0.005			0.023			0.003	
d.f.	21		21		21			21			21	
s.e.d.	11.68		12.43		7.94			3.44			0.33	
l.s.d.	24.28		25.86		16.52			7.15			0.68	
	Significantly different from untreated inoculated control (p>0.05)											
	Not significantly different from untreated inoculated control (p>0.05)											

DT = Duncan test; % Reduction. = % control calculated using Abbott's formula compared to untreated uninoculated. Means are back-transformed.

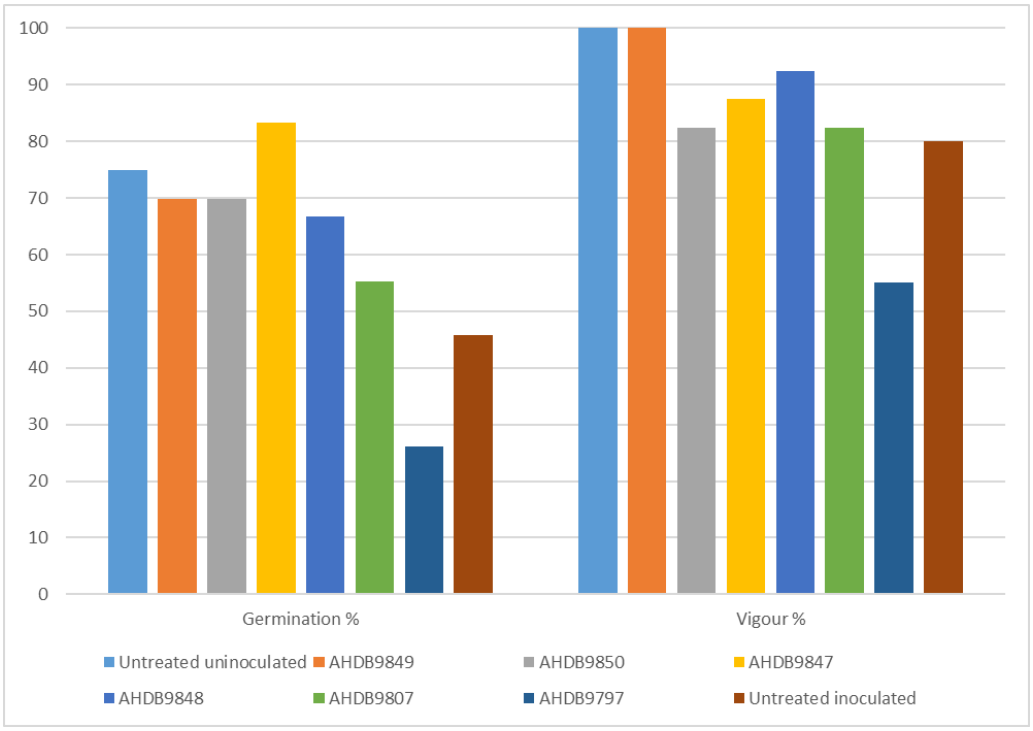

Figure 1. Effect of seed treatment on percentage germination and vigour of seedlings in *R. solani* infested growth media at the last assessment, 23 days after sowing. (n=96 seeds per treatment). Vigour was scored relative to the untreated uninoculated control which was benchmarked at 100%.

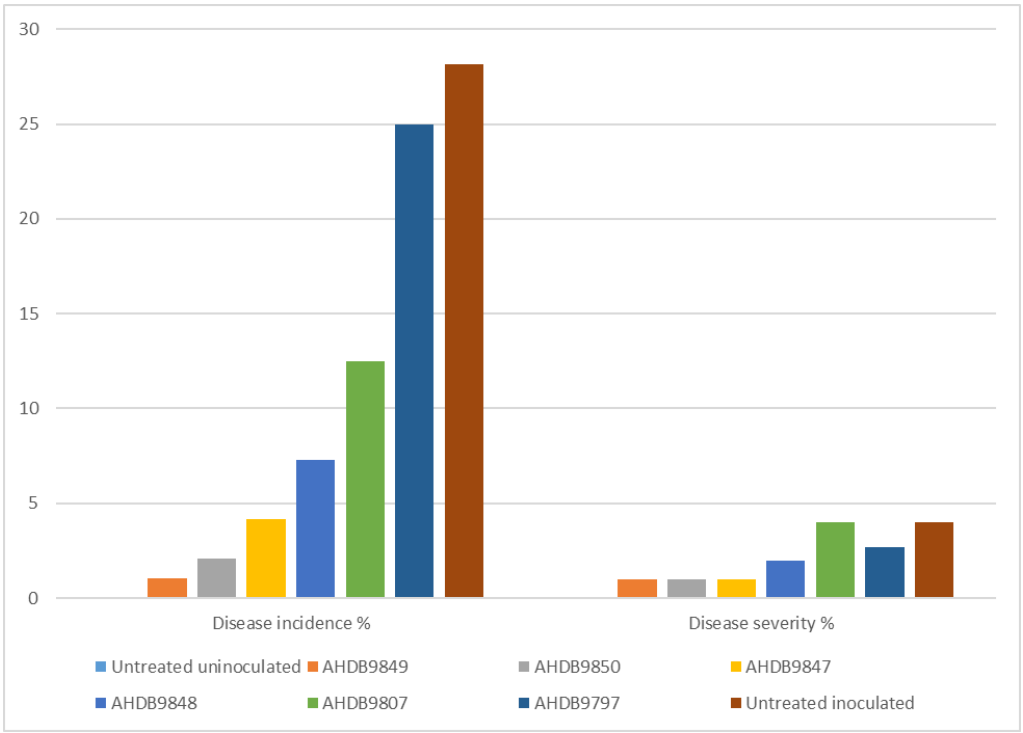

Figure 2. Effect of seed treatment on disease incidence and severity. in *R. solani* infested growth media at the last assessment point, 23 days after sowing. (n=96 seeds per treatment)

Conclusions

Seed toxicity germination test

- Treatment of cauliflower seed with AHDB9849, AHDB9807, AHDB9850, AHDB9847 and AHDB9848 did not adversely affect germination soon after treatment or after an extended storage period.
- AHDB9797 treated seed resulted in very poor germination which could suggest the rate was too high for cauliflower seed.

Seed treatment efficacy for R. solani control

- Treatment with AHDB9847, AHDB9849 and AHDB9850 resulted in the highest seedling emergence rates and very similar levels of disease control with AHDB9848 providing an intermediate level of control.
- AHDB9849 and AHDB9848 resulted in seedling vigour comparable with the untreated uninoculated control. These products may perform better and offer good disease control in the field environment where inoculum load and disease pressure are lower than the experimental condition used.
- AHDB9797 was ineffective at controlling disease but poor germination was most likely to be due to phytotoxicity.

Take home message:

This work identified safe and effective seed treatments which can offer good control against damping off caused by *R. solani*. AHDB9850, AHDB9847 and AHDB9849 are all good alternatives to Thiram which is now unavailable for use. These products contain different actives which is desirable for resistance management strategies. AHDB9849 is a bioprotectant product, based on a strain of *Bacillus*. If an EAMU was granted for this product it would be of benefit to organic growers, as well as reducing chemical fungicide inputs into the soil environment for conventional growers. AHDB9797 performed poorly, but lower rates could be tested in future work to determine if this product is suitable to treat *R. solani* in cauliflower.

Full report

Summary

The fungal plant pathogen *Rhizoctonia solani* causes damping off in cauliflower. The disease is characterized by seed rotting, failed emergence or the death of seedlings soon after they emerge. Seedlings which do emerge develop a lesion at the stem base at the contact point with the soil and this leads to the collapse of the seedling. Some seedlings may manage to grow with the lesion, but in most instances will eventually collapse.

Seed treatment is an important part of managing this disease. Until recently Agrichem Flowable Thiram (thiram) was the industry standard plant protection product used and was applied as a warm water soak. With the withdrawal of this product, alternative seed treatments are required to effectively manage the threat from *R. solani*. Non-chemical alternatives would benefit both conventional and organic growers, given the continued consumer and retailer pressure for a reduction in the use of chemical fungicide products. A trial conducted under controlled environmental conditions was designed to identify potential new seed treatments for cauliflower against *R. solani*.

This trial assessed the efficacy of six products as seed treatments for control of *R. solani* as an alternative to thiram. Germination trials were conducted with treated seed immediately after treatment and after storage for 12 weeks to test phytotoxicity of treatments. Further studies were conducted with treated seeds in *R. solani* infested growth media to test for efficacy of disease control.

Objectives

1. To assess crop safety of seed treatments in cauliflower.
2. To investigate the disease control efficacy of potential new seed treatments against damping off in cauliflower.

Trial conduct

UK regulatory guidelines were followed but EPPO guideline took precedence. The following EPPO guidelines were followed:

EPPO Guideline	Activity	Variation from EPPO
EPPO PP1/135 (4)	Phytotoxicity assessment	None
EPPO PP1/152 (4)	Guideline on design and analysis of efficacy evaluation trials	None
EPPO PP1/225 (2)	Minimum effective dose	None
EPPO PP1/181 (4)	Conduct and reporting of efficacy evaluation trials including good experimental practice	None
EPPO PP 1/214 (3)	Principles of acceptable efficacy	None
PP 1/125 (4)	Seed treatments against seedling diseases (trials under controlled conditions)	None
EPPO PP 1/224 (2)	Principles of efficacy evaluation for minor uses	None

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	Boxworth, Cambridge CB23 4NN
Crop	Cauliflower
Cultivar	Floriade
Soil or substrate type	John Innes No 1
Agronomic practice	N/A
Prior history of site	N/A

Trial design

Three tests were conducted as follows:

1) Phytotoxicity – germination assessment of cauliflower seed 1 and 12 weeks after treatments were applied
2) Determination of *R. solani* inoculum concentration required for efficacy study
3) Disease control efficacy – treated seeds sown in *R. solani* inoculated soil

Germination tests were conducted in controlled environment (CE) cabinets at ADAS, Boxworth.

Phytotoxicity – seed germination

Item	Details
Trial design:	Randomised
Number of replicates:	1
Plot size:	Plastic boxes
Plot size: (cm^2):	201.25 (17.5 x 11.5)
Number of seeds per plot:	100
Number of seeds per treatment:	100

Determination of *R. solani* inoculum concentration

Item	Details
Trial design:	Randomised
Number of replicates:	1
Plot size:	Plastic boxes
Plot size: (cm^2):	201.25 (17.5 x 11.5)
Number of seeds per plot:	60
Number of seeds per treatment:	60

Disease control efficacy trial

Item	Details
Trial design:	Randomised
Number of replicates:	4
Plot size:	Plastic boxes
Plot size: (cm^2):	201.25 (17.5 x 11.5)
Number of seeds per plot:	24
Number of seeds per treatment:	96

Efficacy trial

Table 1. Treatment details

AHDB Code	Active substance	Product name	Company batch number	Content of active substance in product	Product rate (mls or gms/100 kg seed)	Formulation type	Treatment timing
AHDB9797	N/D	N/D	18015/001	N/D	120g per L (10%)	Wettable powder	Seed
AHDB9849	N/D	N/D	0022760086	N/D	1.6 g / Kg seed	Flowable concentrate	Seed
AHDB9807	N/D	N/D	H15507021	N/D	2 ml / Kg seed	Flowable concentrate	Seed
AHDB9850	N/D	N/D	PE-121615M08D015	N/D	1.0 ml / Kg seed	Flowable concentrate	Seed
AHDB9847	N/D	N/D	EGFLO46842 + 1,0	N/D	1.0 ml / Kg seed	Flowable concentrate	Seed
AHDB9848	N/D	N/D	2018-005011	N/D	10 ml / Kg seed	Flowable concentrate	Seed

All treatment seed (including the untreated control) received a polymer film coating of Filmcoat Green as is standard commercial practice.

Table 2. Summary of the trials and treatments conducted in this study

test	Description	Research question	Elsoms	Testing company	Reps	Assessments	Assessment time	Metrics assessed	Total No. seeds
1	Seed toxicity	Does seed treatment affect germination in the absence of the pathogen	Untreated seed	Elsoms	1	1	28 days	Counts: germinated, ungerminated	100
			Filmcoat green						
			AHDB9797						
			AHDB9849						
			AHDB9807	ADAS	1	2	7 and 14 days post-sowing	Counts: germinated, ungerminated. Seedling quality score (1-5)	100
			AHDB9850						
			AHDB9847						
			AHDB9848						
2	Determination of optimal inoculum concentration	What is the optimal concentration of *R solani* in soil to achieve death of 50-90% of seedlings	0, 10%, 25%, 50%, 75%, 90% inoculated growth media	ADAS	1	3	7, 14, 21 days post-sowing	Counts: germinated, ungerminated, dead	60
3	Disease control efficacy	Does seed treatment affect germination in the absence of the pathogen	Untreated seed, uninoculated media	ADAS	4	5	7,13,16,19,23 days	Germination%	96 (4x24)
			Untreated seed, inoculated media					Vigour%	
			AHDB9797				Last assessment only	Disease incidence %, disease severity %, fresh weight	
			AHDB9849						
			AHDB9807						
			AHDB9850						
			AHDB9847						
			AHDB9848						

Methods, assessment and records

Seed preparation

Seed treatment
Approximately 1 kg of untreated cauliflower seed of the susceptible variety Floriade, was obtained from a commercial producer. All seeds were sterilized in the pathology laboratory at ADAS Boxworth (see below) before being send to Elsoms Seeds Ltd. to be coated with the different product treatments. All seeds were also coated with Filmcoat Green as this is commercial standard practice.

Surface sterilization
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.

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 treatment 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	Product rate (mls/g per kg seed)	Application code
1	Untreated	-	A
2	Untreated (Filmcoat Green)	N/K	A
3	AHDB9797	120 or 10%	A
4	AHDB9849	1.6	A
5	AHDB9807	2.0	A
6	AHDB9850	1.0	A
7	AHDB9847	1.0	A
8	AHDB9848	10.0	A

1. Elsoms seed germination tests

This experiment was conducted by Elsoms Seeds Ltd one week after seed treatment (on 26 February 2021).

Each treated seed batch was subsampled for 100 seeds. Moist filter papers were used to line plastic trays and these seeds were sown in a 20 x 5 grid. Trays were covered with lids to prevent moisture loss and incubated in a CE cabinet at 20°C 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 at 28 days with seeds classified and counted as germinated or ungerminated.

2. ADAS seed germination tests

This experiment was set up 12 weeks after seed treatments. Seeds were subsampled for 100 seeds and sown onto filter papers and incubated as above (1).

Seed germination was assessed at 7 and 14 days after sowing and counted in the following categories.

- Germinated seeds
- Ungerminated seeds
- Seedling quality score (1-5)

    1 - Ungerminated dead seed: Seeds which at the end of the test period were either decayed, mouldy or soft.
    2 - Ungerminated viable seed: Seeds which remain firm and apparently viable at the end of the test.
    3 - Germinated with abnormal growth, and roots less than 0.5 cm.
    4 - Germinated with weak growth, and roots 0.5 – 1.0 cm.
    5 - Germinated with normal development: Cotyledons at least 50% emerged with no damage to terminal bud, roots over 1.0 cm.

3. Determination of optimal *R. solani* inoculum rate

Growing media inoculation

To assess the efficacy of the seed treatments as protectants against damping off, cauliflower seeds were sown into soil inoculated with *R. solani*.

Inoculum preparation

Culture preparation

A *R. solani* culture, originally isolated from cauliflower from the ADAS Horticulture culture collection was used for inoculations (isolate code: N/D8). Cultures were removed from storage and subcultured onto Potato Dextrose Agar (PDA), sealed and incubated at 20˚C on an 18:6 light:dark cycle. Cultures were ready to use after approximately 3-7 days or when the mycelial growth had just reached the edge of the plate i.e. actively growing.

Substrate preparation

A 5:3 ratio of medium grade vermiculite:maize meal was mixed and water added and mixed through until completely absorbed to create a uniform medium. The mixture was autoclaved twice for 2 hours at 121˚C.

Substrate inoculation

Three *R. solani* cultures growing in 9 cm petri dishes plates were cut into quarters and added to the surface of the sterile vermiculite:maize substrate in bags and sealed to allow a large air gap above the substrate. Bags were incubated between 20-23˚C in the dark for 3 weeks. During incubation bags were checked for mycelial growth and contamination every 2-3 days and shaken every 3-4 days to distribute mycelium throughout the substrate. After 3 weeks, the inoculum was dried on a paper towel for 5 days in laminar flow cabinets. Once dry the mixture was sieved through a 4 mm sieve to create a free-flowing, homogenous mixture. Inoculum was used immediately.

Growth media preparation

John Innes No. 1 growing media was autoclaved twice for 2 hours at 121˚C to sterilize before use and 200 g dispensed into seed trays. Some sterile media was reserved for use in the uninoculated controls, but the rest was inoculated by mixing the vermiculite inoculum with John Innes No. 1 growth media in the desired ratio. For the trial to determine the optimal rate of *R. solani* inoculum to achieve 50-90% plant death, different mixtures were prepared to give 10, 25, 50, 75 and 90% inoculum concentration in John Innes No. 1.

60 cauliflower seeds were sown into the different concentrations (0/10/25/50/75/90%) of *R. solani* inoculated growth media and incubated at 20˚C with a 16:8 hour light:dark cycle for 28

days. Seed germination was assessed at 7, 14 and 21 days after sowing and the number of emerged seedlings was assessed. The optimal rate of inoculum was defined at that which gives 50% emergence in the inoculated control.

4. Evaluation of seed treatment efficacy

For the disease control efficacy trial, a 10% inoculum mixture was used and made by mixing with the *R. solani* vermiculite inoculum in a 1:9 ratio with the growth substrate.

Seed sowing and incubation
200 g of John Innes No. 1 growing media (uninoculated or inoculated with *R. solani*) was put into each seed tray (17x12 cm). Trays were left for 7 days before sowing seeds.

Four replicates of 24 seeds were sown for each treatment in an 8x3 grid at 2 cm depth. Lids were placed on the seed trays to maintain humidity and the trays were placed in a controlled environment cabinet following a randomized block design.

Seeds were grown in controlled environment cabinets at 20˚C at 80% relatively humidity under a 12:12 light:dark cycle. Once emergence of seedlings had begun, the lids were removed and light was provided.

At each assessment point the were assessed as follows:

- Number of emerged seedlings.
- Number of seedlings failing to emerge.
- Seedling vigour % - assess relative to uninoculated control which sets the 100% vigorous benchmark to assess against. Missing plants were not included.

At the final assessment the following additional measures were assessed:

- Disease incidence (percentage plants affected)
- Disease severity score
    1. Healthy 0% symptoms
    2. 1-25% roots with symptoms
    3. 26-50% roots with symptoms
    4. 51-75% roots with symptoms
    5. 76-100% roots with symptoms

  Note: All non-emerged plants are considered to have been killed by the pathogen and are recorded as 5.
- Fresh weight – recorded at the end of the trial. All plants are gently washed, blotted and weighed.

Assessment Schedule

Assessment no.	Assessment date	Seedling emergence in the uninoculated control (%)	Seedling emergence in the inoculated control (%)
1	12.05.21	66.7	22.2
2	18.05.21	72.9	30.2
3	21.05.21	75.0	34.4
4	24.05.21	76.0	45.8
5	28.05.21	75.0	78.1

Statistical analysis
The germination tests and efficacy trial were laid out as a randomised complete block design. Statistical analysis was carried using ANOVA with a Duncan's Multiple Range Test in Genstat 18. To assess for differences between treatments compared to the untreated control, germination, vigour, disease incidence, severity and fresh weight values were used as variables to determine efficacy. All percentage data was transformed with an angular transformation. Back transformed means are reported in results Table 5. Abbott's formula was also used to calculate the percentage change for each treatment relative to the control.

Abbott's formula = Percent reduction in control = (% alive in the check - % alive in the treatment/%alive in the treatment) x 100.

Results

1. Elsom's seed germination trial

This assessment was conducted by Elsoms Seeds Ltd., one month after seed treatment (26 February 2021). Germination was scored as the percentage of seeds that led to viable growing seedlings after a month. Seedling quality and vigour were not recorded.

Table 3. Germination of seeds 1 month after treatment.

Treatment code	Treatment	Elsoms lot no.	Germination % final
1	Untreated	E71108	97
2	Filmcoat Green	E71158	95
3	AHDB9797	E71159	46
4	AHDB9849	E71160	95
5	AHDB9807	E71162	95
6	AHDB9850	E71164	95
7	AHDB9847	E71167	95
8	AHDB9848	E71168	96

All treatments, except for AHDB9797 had very high germination rates (Table 3). Replicate results were not provided and so statistical comparison was not done. The untreated control resulted in the best results with 97% germination, but was closely followed by the other treatments which ranged between 95% (Untreated + Filmcoat Green, AHDB9849, AHDB9807, AHDB9850, AHDB9847) and 96% (AHDB9848). The AHDB9797 treatment had just 46% germination indicating that the treatment at this application rate has a detrimental effect on germination.

2. ADAS seed germination trial

This trial was conducted 12 weeks after seed treatment. Seeds were scored as germinated/ungerminated at two timepoints (7 and 14 days) and scored for seedling quality on a 1-5 scale. Untreated seed which were not coated in Filmcoat Green were not included in this trial.

As was observed in the Elsoms germination trial, all treatments with the exception AHDB9797 showed very high germination rates (Table 4). At 14 days after sowing 7 out of 8 treatments were at 100% germination, while AHDB9797 had just 28 out of 100 seeds germinated. This was a decrease in germination rate for AHDB9797 compared with the earlier trial at Elsoms, where 46 seeds out of 100 germinated.

Seedling quality was also very high with scores of 5 in all but AHDB9797 at the last assessment, indicating very healthy seedling development and no adverse effects of seed treatment on plant quality. At the 7 day assessment, seeds treated with AHDB9850 were scored as 4 due to shorter roots and some light browning on the root tips, which were not observed in other treatments. Lateral roots were also absent on the majority of AHDB9850 treated plants. Cotyledons were noticeably smaller and paler in colour and plants less vigorous. However, at the 14 day assessment the roots had developed to a comparable level with other treatments i.e. laterals had developed. Cotyledons had also developed similar vigour and colouration as the untreated control. This suggests that AHDB9850 may cause a slight lag in development but have no longer term effects.

AHDB9797 seedling quality was poor at 7 days with most seeds ungerminated or with visible mould growing on the surface. At 14 days those that had fully emerged had abnormal colour, shape and orientation.

Table 4. Longer term storage of seeds (4 months). Germination and seedling quality at 7 and 14 days after sowing.

Treatment	Germination % 7 d	Seedling quality (1-5 index)	Germination % 14 d	Seedling quality (1-5 index)
Untreated	100	5	100	5
AHDB9797	8	2	28	3
AHDB9849	100	5	100	5
AHDB9807	100	5	100	5
AHDB9850	98	4	100	5
AHDB9847	97	5	100	5
AHDB9848	100	5	100	5

3. Determination of optimal inoculum concentration

Untreated cauliflower seeds were sown into growth medium inoculated with different concentrations of *R. solani*. All trays with *R. solani* inoculated soil resulted in a reduction in germination or in the case of the 75% and 100% inoculum rates no germination was recorded at all. For the higher inoculum concentrations (50/75/90%) the growth medium became partially overgrown with pathogen mycelium by 14 days (Figure 1). Based on this initial trial, an inoculum rate of 10% v/v was determined to be the optimal concentration to use for the treated seed germination trials as it resulted in disease but still allowed 25% emergence rate at 14 days, which was reduced to 15% at 21 days. This level of disease pressure was considered to potentially allow differences to be observed between treatments in subsequent efficacy tests.

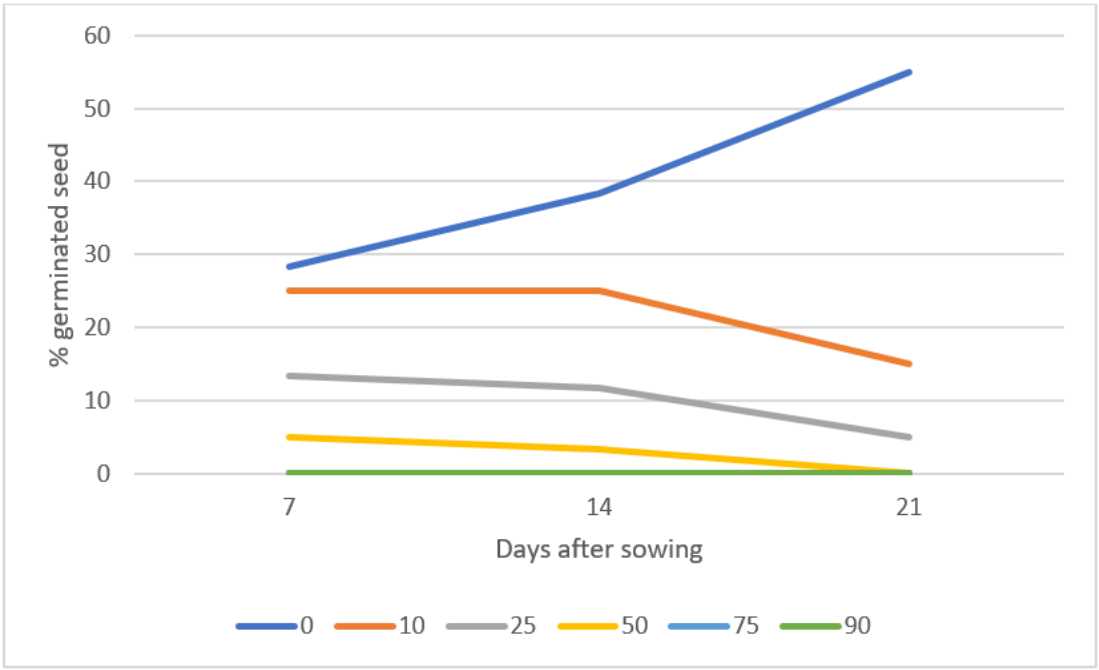

Figure 1. Percentage germination of cauliflower seeds sown into different *R. solani* inoculum concentrations (0/10/25/50/75/90%) in John Innes No. 1 growth medium assessed at 7, 24 and 21 days after sowing.

Seed treatment disease control efficacy trial

Seedling emergence for the untreated uninoculated control treatment was 76.7% at the final assessment 23 days after sowing. As expected, the untreated seed in inoculated soil had a low

germination rate (45.6%) indicating that the disease pressure was sufficient to cause damping off but not so extreme that any effect of treatment would be overcome.

All treatments, apart from AHDB9797 had higher emergence rates than the untreated inoculated control (45.6%) (Figures 2 and 4, Table 5). AHDB9847 had the highest emergence rate (84.6%), which exceeded the untreated uninoculated control (76.7%). Although the trend was for treatments to improved performance, the differences in emergence between products were not significantly different from the untreated inoculated control (p=0.089). AHDB9797 did however impact emergence with only 25.7% of seedlings emerging. This may have been the result of phytotoxicity of the treatment (see germination trial results above), rather than disease susceptibility and may have performed better with a reduced coating rate.

Table 5. Effect of plant protection products on average germination, vigour, disease incidence, severity and the average sum of fresh weight of cauliflower seed at final assessment point 23 days after sowing. 4 replicates per treatment (4 x 24, n=96).

Treatment	Emerged %	DT	Vigour %	DT	Disease incidence %	DT	% control	Disease severity %	DT	% control	Fresh weight g	DT
Untreated uninoculated	76.7	b	100.0	b	0.00	a	100	0.00	a	100	1.95	d
Untreated inoculated	45.6	ab	89.0	ab	25.92	d	0	3.74	c	0	0.71	ab
AHDB9797	25.7	a	55.0	a	19.81	cd	23.6	2.26	bc	39.6	0.34	a
AHDB9849	72.8	b	100.0	b	0.26	ab	99.0	0.25	ab	93.3	1.05	abc
AHDB9807	54.6	ab	90.6	ab	11.89	bcd	54.1	4.00	c	-7.0	0.98	abc
AHDB9850	72.1	b	90.3	ab	0.54	ab	97.9	0.25	ab	93.3	1.36	bcd
AHDB9847	84.6	b	93.8	ab	2.00	ab	92.28	0.25	ab	93.3	1.547	cd
AHDB9848	73.5	b	97.9	b	3.63	abc	86.0	1.01	abc	73.0	1.1	abc
P value	0.089		0.062		0.005			0.02			0.003	
d.f.	21		21		21			21			21	
s.e.d.	11.68		12.43		7.94			3.44			0.33	
l.s.d.	24.28		25.86		16.52			7.15			0.68	
	Significantly different from untreated inoculated control (p>0.05)											
	Not significantly different from untreated inoculated control (p>0.05)											

DT = Duncan test; % Reduction. = % control calculated using Abbott's formula compared to untreated uninoculated. Means are back-transformed.

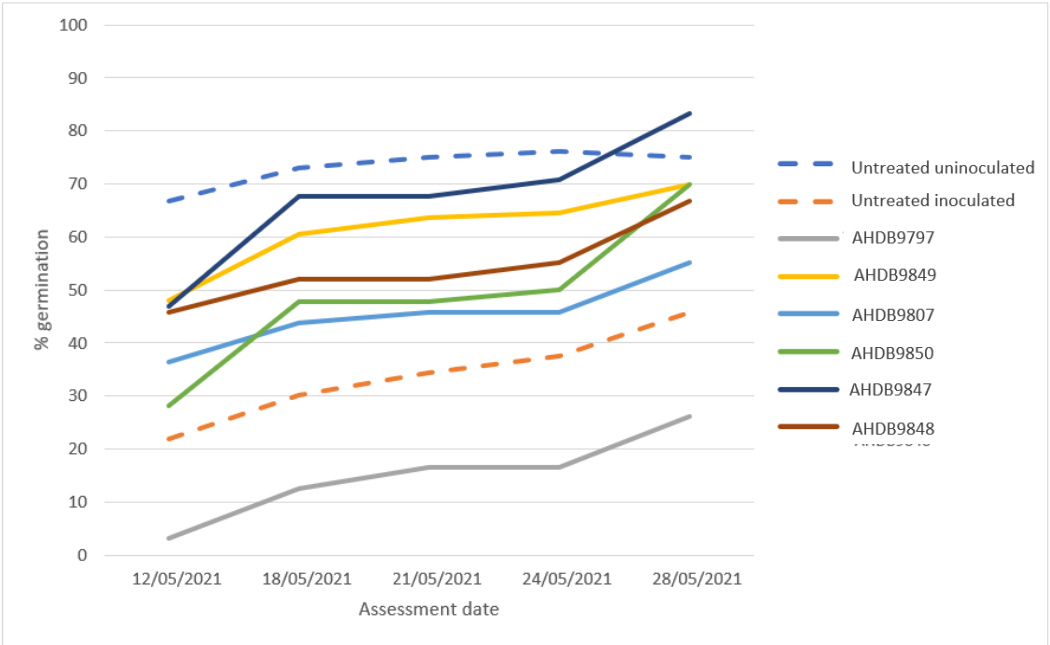

Figure 2. Effect of seed treatment on seed germination (%) in *R. solani* infested growth media over time. (n=96 seeds per treatment)

Seed vigour was assessed using the untreated uninoculated control as a 100% benchmark. All treatments, with the exception of AHDB9797, had comparable vigour ratings when compared with the untreated inoculated control (89%). AHDB9849 was the best performer (100%) which exceeded the untreated inoculated control and matched that of the untreated uninoculated control. However, there were no significant differences between treatments (p=0.062) (Figures 3 and 4, Table 5).

As with emergence, the low vigour score of AHDB9797 (55%) was likely due to phytotoxic effects of the treatment rather than disease pressure and was significantly different from both inoculated and uninoculated untreated controls (Duncan test p=0.05).

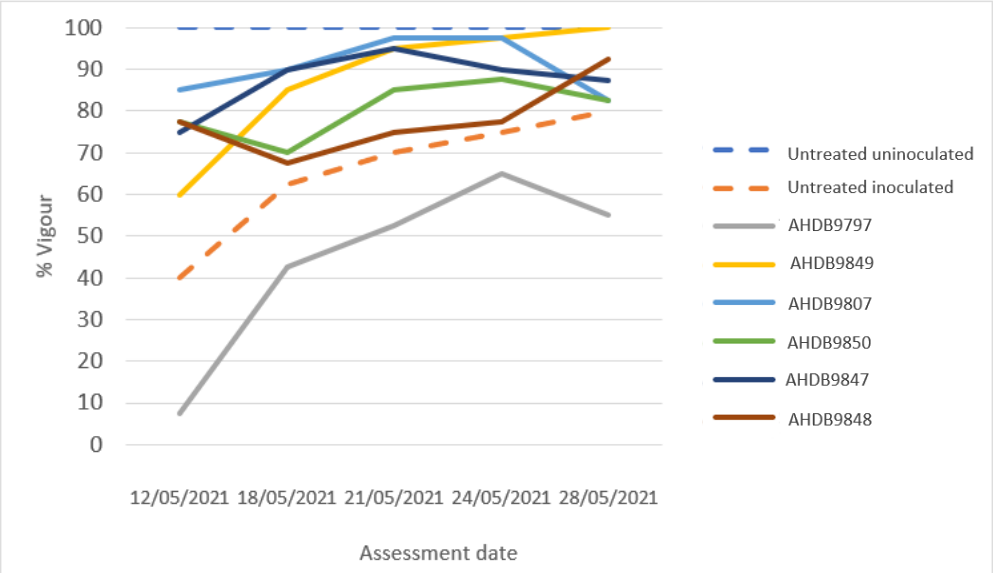

Figure 3. Effect of seed treatment on seedling vigour (%) in *R. solani* infested growth media over time. (n=96 seeds per treatment) Vigour was assessed relative to the untreated uninoculated control which was the 100% benchmark.

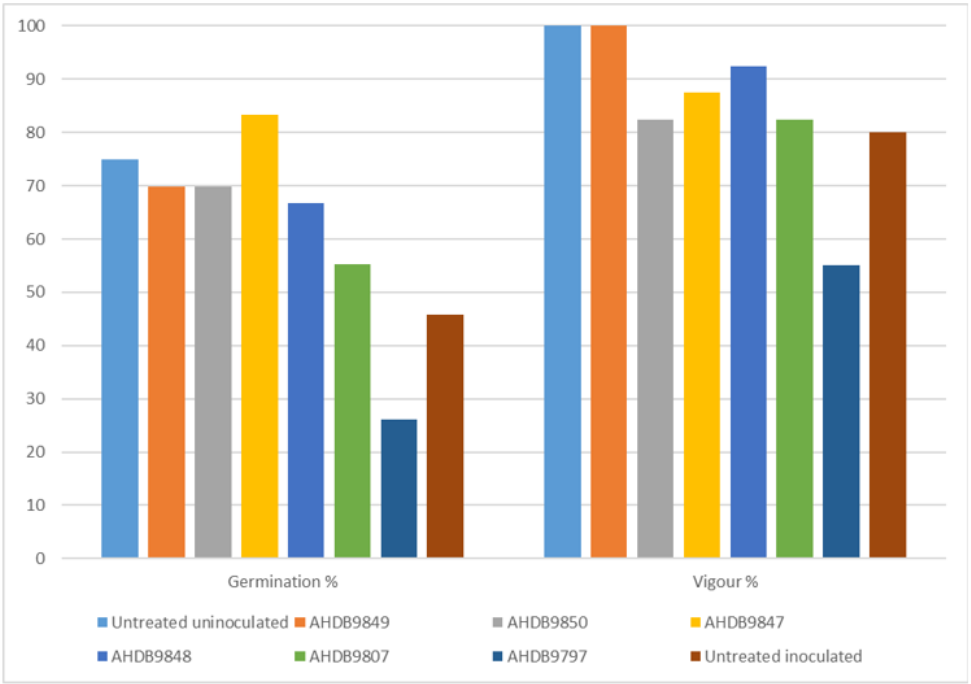

Figure 4. Effect of seed treatment on percentage germination and vigour of seedlings in *R. solani* infested growth media at the last assessment point, 23 days after sowing. (n=96 seeds per treatment). Vigour was scored relative to the untreated uninoculated control which was benchmarked at 100%.

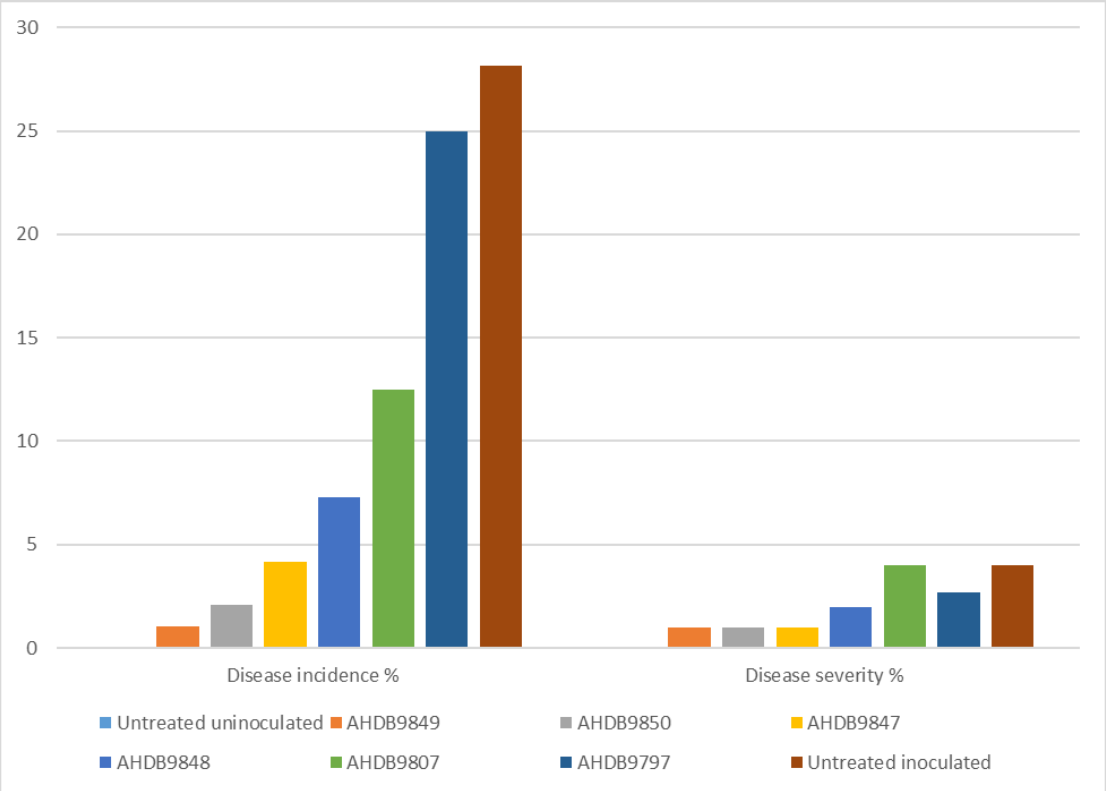

Figure 5. Effect of seed treatment on disease incidence and severity. in *R. solani* infested growth media at the last assessment point, 23 days after sowing. (n=96 seeds per treatment)

Disease incidence and severity was greatest in the untreated inoculated treatment and 0.0% in the uninoculated control as might be expected (Figure 5). There was a significant difference between treatments for disease incidence (p=0.003) and severity (p=0.023, Table 5). Disease incidence was lowest for AHDB9849 (0.3%), followed by AHDB9850 (0.5%), AHDB9847 (2.0%) and AHDB9848 (3.6%) which were not significantly different from each other (Duncan's test, Table 3). Disease incidence for AHDB9797 (19.8%) and AHDB9807 (11.9%) was not significantly different from the untreated inoculated control treatment.

The results for disease incidence were mostly confirmed for measurements of disease severity (Figure 4) except for AHDB9807 which had more severe disease than AHDB9797. Although there was a significant difference between treatments for disease severity, the Duncan's test showed that these differences were mainly between the treatments and the uninoculated control with the treatments all performing similarly (Table 5). Abbott's formula was calculated to quantify the % control of disease compared to the untreated control. AHDB9849, AHDB9850, AHDB9847 resulted in 92 and 99% disease control for incidence and this was similar for disease severity.

Seedling fresh weight was also significantly different between treatments (p=0.003, Table 5). As expected, the untreated uninoculated control had the greatest average fresh weight (1.95 g). AHDB9847 performed best in this metric with an average sum of fresh weight of 1.55 g which was significantly greater than the fresh weight in the untreated inoculated control, but not significantly different from the untreated uninoculated control. The other treatments were not significantly different from one another or the untreated inoculated control despite the trend for greater fresh weight when treated.

Conclusions

Germination seed phytotoxicity

- All treatments apart from AHDB9797 did not adversely affect germination rates immediately after or following 12 weeks of storage.
- AHDB9797 treated seed had a very poor germination rate at both timepoints. It is possible that the rate of this product was too high for cauliflower seeds and further work could be done to determine a safe rate.

Optimal inoculum rate

- The final *R. solani* inoculum rate that was chosen for further studies was 10% and provided a good level of disease pressure to challenge the treated seeds. However, this rate is probably in excess of what natural inoculum loads would be in a growing field. Those treatments that offered some disease control but did not perform as well as others could still offer good disease control in low inoculum areas. Further work could include testing seed treatments at lower levels of inoculum.

Seed treatment efficacy for *R. solani* control

- AHDB9849, AHDB9850 and AHDB9847 resulted in very similar levels of disease control both in terms of incidence and severity. AHDB9847 and AHDB9850 belong to different Fungicide Resistance Action Committee (FRAC) groups, 3 and 12 respectively. These offer effective, crop safe alternatives to thiram which could be incorporated into integrated pest and disease management (IPDM) strategies, a valuable tool in fungicide resistance management. AHDB9849, a bioprotectant (FRAC BM02) based on a *Bacillus* strain gave comparable results to both conventional products and would be of particular use to organic cauliflower producers if an EAMU was granted.
- AHDB9848 and AHDB9807 provided an intermediate level of disease control. This experiment was conducted under relatively high disease pressure conditions in the lab and hence these products may offer better control when challenged with lower inoculum rates in the field. AHDB9797 did not provide good control, but this could be due to seed phytotoxicity. Further work could explore lower rates.

Appendix

a. Crop diary – events related to growing crop

Crop	Cultivar	Treatment date
Cauliflower	Floriade	19.02.2021

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

Date	Event
Optimal load tests	
29/09/2020	Trial set-up
16/10/2020	Seedling emergence (germination) assessment
Germination tests - Elsoms Seeds Ltd.	
26/02/2021	Germination trial set-up
Efficacy tests	
30.04.2021	Soil artificially inoculated with *F. culmorum* and placed in plastic trays

05.05.2021	Efficacy trial established in the ADAS Pathology laboratory controlled environment cabinets
12.05.2021	Assessment 1: Seedling emergence (germination), disease incidence, severity and vigour.
18.05.2021	Assessment 2: Seedling emergence (germination), disease incidence, severity and vigour.
21.05.2021	Assessment 3: Seedling emergence (germination), disease incidence, severity and vigour.
24.05.2021	Assessment 4: Seedling emergence (germination), disease incidence, severity and vigour.
28.05.2021	Assessment 5: Seedling emergence (germination), disease incidence, severity, vigour and fresh weight.
Germination tests representing commercial storage – ADAS	
10.05.2021	Germination trial set-up
17.05.2021	Germination assessment 1
24.05.2021	Germination assessment 2

c. Raw data from assessments

- Phytotoxicity - germination tests following seed treatment – Test conducted by Elsoms

Treatment code	Treatment	Lot no.	Germination % final
1	Untreated	E71108	97
2	Filmcoat green	E71158	95
3	AHDB9797	E71159	46
4	AHDB9849	E71160	95
5	AHDB9807	E71162	95
6	AHDB9850	E71164	95
7	AHDB9847	E71167	95
8	AHDB9848	E71168	96

- Phytotoxicity - germination tests 12 weeks after of long term storage of treated seed

Treatment	Germination 7 d	Seedling quality (1-5)	Germination 14 d	Seedling quality (1-5)
Filmcoat green	100	5	100	5
AHDB9849	100	5	100	5
AHDB9847	97	5	100	5
AHDB9807	100	5	100	5
AHDB9850	98	4	100	5
AHDB9848	100	5	100	5
AHDB9797	8	2	28	3

Efficacy trial - seed germination in inoculated soil data

Plot	Block	Trt no.	Treatment	Germ % 12.05	Germ % 18.05	Germ % 21.05	Germ % 24.05	Germ % 28.05	Ungerm % 12.05	Ungerm % 18.05	Ungerm % 21.05	Ungerm % 24.05	Ungerm % 28.05
101	1	2	Untreated inoculated	4.2	4.2	4.2	4.2	4.2	95.8	95.8	95.8	95.8	95.8
102	1	8	AHDB9848	62.5	75.0	75.0	79.2	83.3	37.5	25.0	25.0	20.8	16.7
103	1	3	AHDB9797	0.0	4.2	4.2	4.2	16.7	100.0	95.8	95.8	95.8	83.3
104	1	4	AHDB9849	8.3	8.3	8.3	8.3	8.3	91.7	91.7	91.7	91.7	91.7
105	1	5	AHDB9807	4.2	4.2	4.2	4.2	8.3	95.8	95.8	95.8	95.8	91.7
106	1	1	Untreated uninoculated	50.0	54.2	54.2	58.3	58.3	50.0	45.8	45.8	41.7	41.7
107	1	6	AHDB9850	4.2	4.2	4.2	8.3	33.3	95.8	95.8	95.8	91.7	66.7
108	1	7	AHDB9847	8.3	12.5	12.5	20.8	70.8	91.7	87.5	87.5	79.2	29.2
201	2	2	Untreated inoculated	0.0	20.8	33.3	41.7	58.3	100.0	79.2	66.7	58.3	41.7
202	2	3	AHDB9797	4.2	12.5	20.8	20.8	20.8	95.8	87.5	79.2	79.2	79.2
203	2	5	AHDB9807	75.0	83.3	83.3	83.3	79.2	25.0	16.7	16.7	16.7	20.8
204	2	4	AHDB9849	87.5	87.5	87.5	87.5	91.7	12.5	12.5	12.5	12.5	8.3
205	2	7	AHDB9847	16.7	87.5	87.5	87.5	87.5	83.3	12.5	12.5	12.5	12.5
206	2	1	Untreated uninoculated	87.5	87.5	91.7	91.7	91.7	12.5	12.5	8.3	8.3	8.3
207	2	8	AHDB9848	91.7	95.8	95.8	95.8	100.0	8.3	4.2	4.2	4.2	0.0
208	2	6	AHDB9850	29.2	83.3	83.3	83.3	87.5	70.8	16.7	16.7	16.7	12.5
301	3	1	Untreated uninoculated	91.7	91.7	91.7	91.7	87.5	8.3	8.3	8.3	8.3	12.5
302	3	7	AHDB9847	87.5	95.8	95.8	95.8	95.8	12.5	4.2	4.2	4.2	4.2
303	3	5	AHDB9807	4.2	4.2	4.2	4.2	50.0	95.8	95.8	95.8	95.8	50.0
304	3	3	AHDB9797	8.3	16.7	20.8	20.8	33.3	91.7	83.3	79.2	79.2	66.7
305	3	8	AHDB9848	12.5	16.7	16.7	20.8	29.2	87.5	83.3	83.3	79.2	70.8
306	3	2	Untreated inoculated	8.3	8.3	8.3	12.5	25.0	91.7	91.7	91.7	87.5	75.0
307	3	4	AHDB9849	87.5	91.7	100.0	100.0	95.8	12.5	8.3	0.0	0.0	4.2
308	3	6	AHDB9850	8.3	16.7	16.7	16.7	66.7	91.7	83.3	83.3	83.3	33.3

Plot	Block	Trt no.	Treatment	Germ % 12.05	Germ % 18.05	Germ % 21.05	Germ % 24.05	Germ % 28.05	Ungerm % 12.05	Ungerm % 18.05	Ungerm % 21.05	Ungerm % 24.05	Ungerm % 28.05
401	4	3	AHDB9797	0.0	16.7	20.8	20.8	33.3	100.0	83.3	79.2	79.2	66.7
402	4	8	AHDB9848	16.7	20.8	20.8	25.0	54.2	83.3	79.2	79.2	75.0	45.8
403	4	7	AHDB9847	75.0	75.0	75.0	79.2	79.2	25.0	25.0	25.0	20.8	20.8
404	4	4	AHDB9849	8.3	54.2	58.3	62.5	83.3	91.7	45.8	41.7	37.5	16.7
405	4	2	Untreated inoculated	75.0	87.5	91.7	91.7	95.8	25.0	12.5	8.3	8.3	4.2
406	4	6	AHDB9850	70.8	87.5	87.5	91.7	91.7	29.2	12.5	12.5	8.3	8.3
407	4	5	AHDB9807	62.5	83.3	91.7	91.7	83.3	37.5	16.7	8.3	8.3	16.7
408	4	1	Untreated uninoculated	37.5	58.3	62.5	62.5	62.5	62.5	41.7	37.5	37.5	37.5

Plot	Block	Trt no.	Treatment	Vigour % 12.05	Vigour % 18.05	Vigour % 21.05	Vigour % 24.05	Vigour % 28.05	Incidence % 28.05	Severity % 28.05	Fresh weight (g) 28.05
101	1	2	Untreated inoculated	30	80	90	100	100	41.7	4	0.16
102	1	8	AHDB9848	100	100	100	100	100	0.0	0	1.30
103	1	3	AHDB9797	0	20	60	70	70	0.0	0	0.14
104	1	4	AHDB9849	50	80	100	100	100	4.2	4	0.16
105	1	5	AHDB9807	100	90	100	100	80	12.5	4	0.22
106	1	1	Untreated uninoculated	100	100	100	100	100	0.0	0	1.33
107	1	6	AHDB9850	100	90	100	100	70	8.3	4	0.23
108	1	7	AHDB9847	110	110	100	70	60	12.5	4	1.07
201	2	2	Untreated inoculated	0	20	20	30	40	29.2	3	0.57
202	2	3	AHDB9797	10	80	70	80	60	33.3	4	0.34
203	2	5	AHDB9807	80	100	100	100	100	4.2	4	1.11
204	2	4	AHDB9849	80	100	100	100	100	0.0	0	0.85
205	2	7	AHDB9847	60	80	100	100	100	4.2	0	1.15
206	2	1	Untreated uninoculated	100	100	100	100	100	0.0	0	2.30
207	2	8	AHDB9848	80	50	60	70	100	0.0	0	1.30

Plot	Block	Trt no.	Treatment	Vigour % 12.05	Vigour % 18.05	Vigour % 21.05	Vigour % 24.05	Vigour % 28.05	Incidence % 28.05	Severity % 28.05	Fresh weight (g) 28.05
208	2	6	AHDB9850	90	70	100	100	100	0.0	0	1.18
301	3	1	Untreated uninoculated	100	100	100	100	100	0.0	0	1.82
302	3	7	AHDB9847	70	90	90	100	100	0.0	0	2.17
303	3	5	AHDB9807	60	70	90	90	50	16.7	4	0.49
304	3	3	AHDB9797	20	50	60	60	60	37.5	4	0.58
305	3	8	AHDB9848	80	80	90	90	100	8.3	4	0.89
306	3	2	Untreated inoculated	60	80	90	90	80	37.5	4	0.47
307	3	4	AHDB9849	90	90	100	100	100	0.0	0	1.23
308	3	6	AHDB9850	50	40	60	60	60	0.0	0	1.76
401	4	3	AHDB9797	0	20	20	50	30	29.2	4	0.30
402	4	8	AHDB9848	50	40	50	50	70	20.8	4	0.79
403	4	7	AHDB9847	60	80	90	90	90	0.0	0	1.80
404	4	4	AHDB9849	20	70	80	90	100	0.0	0	1.94
405	4	2	Untreated inoculated	70	70	80	80	100	4.2	4	1.63
406	4	6	AHDB9850	70	80	80	90	100	0.0	0	2.26
407	4	5	AHDB9807	100	100	100	100	100	16.7	4	2.10
408	4	1	Untreated uninoculated	100	100	100	100	100	0.0	0	2.36

d. Photos

i) Inoculum load test 0% *R. solani.,* ii), inoculum load test 10% *R. solani.,* iii), efficacy trial in controlled environment cabinets (pre-germination)., iv) Seedling emergence differences between untreated uninoculated (left) vs. untreated inoculated (right).

e. 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 Alison Richardson
Authorised signatory

Certification Number
ORETO 409

HSE
Chemicals Regulation Division

Department of Agriculture and Rural Development