SCEPTREPLUS

Final Trial Report

Trial code:	SP62b
Title:	Evaluation of new seed treatments for control Fusarium in leek
Crop:	Field vegetables - Leek
Targets:	Leek Fusarium, *Fusarium oxysporum* f. sp*. cepae* (FUSACE) and *F. culmorum* (FUSACU)
Lead researcher:	Dave Kaye (ADAS)
Organisation:	RSK ADAS Ltd.
Period:	July 2021 - September 2021
Report date:	30th September 2021
Report author:	Dave Kaye
Report authorised by:	Catherine Eyre
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: 30 September 2021 Authors signature: D. Kaye.

Grower Summary

Introduction

The fungal pathogens *Fusarium culmorum* and *Fusarium oxysporum* f. sp*. cepae* are both known to cause damping off in leek, which causes emerging seedlings to collapse, often submerged in a mass of white fungal growth. Fusarium is one of the most destructive diseases of field-grown leek crops, with inoculum overwintering on crop debris in the soil. Survival in this manner enables Fusarium to infect newly developing seedlings.

Seed treatment remains an important component of Fusarium management. However, effective control is threatened by the recent loss of thiram, the standard seed treatment, which was applied as a warm water soak. Non-chemical alternatives to thiram would be of major benefit to both organic and conventional growers, given continued consumer and retailer pressure for a reduction in the use of chemical fungicide products.

Potential new seed treatments were trialed against Fusarium damping off under controlled environmental conditions and artificial inoculation with *F. culmorum* to determine if any could provide an alternative to thiram, for both conventional and organic leek production.

Methods

Crop safety (phytotoxicity) and disease control efficacy experiments were conducted with leek seed of the *F. culmorum* susceptible variety Musselborough. Seeds were subsampled and treated with 7 different seed fungicide and bioprotectant treatments by Elsoms Seeds Ltd.

Phytotoxicity

The effect of the seed treatments on germination and seedling quality was assessed in two experiments, where seeds were sown onto moist filter paper in sealed plastic boxes either (i) one week after treatment or (ii) after 5 weeks of storage. Seed from each treatment, as well as the untreated sample, were stored at ADAS Boxworth under refrigerated conditions (4-8ºC, darkness). Germinated seeds were counted every 7 days for 28 days, with seedling/root quality and total plot fresh weight recorded at day 28.

Data was recorded using counts of seeds in the following categories:

- Germinated with normal development: cotyledons at least 50% emerged with no damage to terminal bud, roots > 1.0 cm.
- Germinated with weak growth and roots 0.5 – 1.0 cm.
- Germinated with abnormal growth and roots < 0.5 cm.
- Ungerminated viable seed: seed which remained firm and apparently viable at the end of the test.
- Ungerminated dead seed: seed which at the end of the test period were either decayed, mouldy or soft.
- Total plot fresh weight (g) – destructive assessment, recorded at last assessment (28 d). Plant gently blotted to remove moisture.

Seed treatment efficacy for control of *F. culmorum*

Growing media (John Innes no. 1) was artificially inoculated with a known pathogenic isolate of *F. culmorum* at $1x10^4$ spores/g growing media. This spore concentration was determined in preliminary experiments to give good disease pressure. 400 g of the inoculated growing media was placed in plastic trays (17 x 13 cm) and incubated for 7 days in controlled environment (CE) cabinets at 20ºC at 80% relatively humidity under an 18:6 light:dark cycle. Following incubation, 24 treated (see table below) or untreated leek seed were sown in a 3 x 8 grid pattern at a depth of 2 cm, with lids placed on the boxes. These were returned to the CE cabinets and the lids removed following seedling emergence.

Seedling assessments began 14 days after sowing (d.a.s.) shortly after emergence had started and were conducted twice a week, until the final assessment at 26 d.a.s. An untreated uninoculated control was run alongside an untreated inoculated control to confirm the pathogenicity of the *F. culmorum* isolate and to compare with treatment effects.

Disease levels caused by all of the treatments was determined by assessment of the following:

- Seedling emergence counts.
- Fusarium incidence: the number of seedlings which collapsed after emergence due to infection, as well as the presence of *F. culmorum* (white fungal growth) on seedlings.

Results

Seed toxicity germination test

Germination immediately after treatment

- All treatments resulted in very high germination rates of 89-93% (Table 1).
- No significant differences in the number of healthy seedlings (those germinated with normal development) developed between treated and untreated seeds at the 28 day destructive assessment.
- Significantly fewer (p=0.005) seedlings classed as weak were recorded in seed treated with AHDB9849 (2.5%), AHDDB9807 (1.0%) and AHDB9848 (1.5%) compared with the untreated control (6.5%).
- No differences in the number of abnormal seed (p=0.771), viable seed (p=0.052) or dead seed (p=0.385) were present between treated and untreated seed.

Table 1. Effect of treatments on leek seed germination and the quality of seedling development 28 days after sowing – germination trial immediately after storage, 02 August 2021

Treatment	Overall germination (%)	Mean number seeds germinated (%)			Mean number of ungerminated seeds (%)	
		Normal	Weak	Abnormal	Viable seed	Dead seed
Untreated	92.0	82.5	6.5	3.0	5.0	3.0
AHDB9734	90.5	81.0	7.5	2.0	2.0	7.5
AHDB9849	89.0	84.0	2.5	2.5	1.0	10.0
AHDB9807	89.0	83.5	1.0	4.5	4.0	7.0
AHDB9850	91.0	85.5	3.5	2.0	2.5	6.5
AHDB9847	91.5	84.5	4.0	3.0	2.5	6.0
AHDB9763	93.0	89.0	3.0	1.0	1.0	6.0
AHDB9848	90.5	87.5	1.5	1.5	2.5	7.0
	Significantly different from the untreated control (p<0.05)					
	Not significantly different from the untreated control (p>0.05)					

Germination 5 weeks after treatment

- Germination rates remained high after 5 weeks of seed storage for all treatments, between 87 and 92% (Table 2).
- No significant differences in the number of healthy seedlings (those germinated with normal development) developed between treated and untreated seeds at the 28day destructive assessment.
- The number of normal, weak or abnormal seedlings or the number of ungerminated seeds (viable or dead) was comparable and not significantly different from the compared with the untreated control.

Table 2. Effect treatments on on leek seed germination and the quality of seedling development at 28 days after sowing – germination trial after 5 weeks seed storage, 06 September 2021

Treatment	Overall Germination (%)	Mean number seeds germinated (%)			Mean number of seeds not germinated (%)	
		Normal	Weak	Abnormal	Viable seed	Dead seed
Untreated	89.5	83.0	6.0	0.5	4.0	6.5
AHDB9734	91.5	86.0	5.0	0.5	2.5	6.0
AHDB9849	87.5	79.5	4.5	3.5	3.5	9.0
AHDB9807	87.0	80.0	4.5	2.5	5.5	7.5
AHDB9850	86.5	80.0	4.5	2.0	3.5	10.0
AHDB9847	89.5	85.0	4.0	0.5	4.5	6.0
AHDB9763	88.0	84.5	3.0	0.5	3.5	8.5
AHDB9848	87.0	73.0	10.0	4.0	6.0	7.0
	Significantly different from the untreated control (p<0.05)					

	Not significantly different from the untreated control (p>0.05)

Seed treatment efficacy
Emergence

- Untreated seed in *F. culmorum* inoculated soil had a lower emergence rate compared with that of the untreated seed grown in uninoculated soil (66.7% vs. 92.7% at 26 d.a.s.).
- Emergence of the untreated uninoculated control seedling was 92.7% at the final assessment, 26 days after sowing (Table 3, Figure 1).
- All treatments, apart from AHDB9807 significantly increased seedling emergence compared with the untreated inoculated control at every assessment date.
- At the final assessment (26 d.a.s.) seedling emergence was greatest in seed treated with AHDB9849 (90.6%), AHDB9850 (90.6%) and AHDB9847 (86.5%) compared with the untreated inoculated (66.7%) and AHDB9807 (76.0%, p=0.001).
- No significant differences were recorded between AHDB9734. AHDB9849, AHDB9850, AHDB9847. AHDB9763, AHDB9848 and the uninoculated untreated control at the final assessment (26 d.a.s.).

Table 3. Effect of treatments on mean seedling emergence incidence (%) for each of five assessment dates.

Treatment	% emergence				
	21/07/21 14 d.a.s.	23/07/21 16 d.a.s.	26/07/21 19 d.a.s.	30/07/21 23 d.a.s.	02/08/21 26 d.a.s.
Untreated uninoculated	90.63	90.63	91.67	91.67	92.71
Untreated inoculated	67.71	66.67	66.67	66.67	66.67
AHDB9734	82.29	82.29	82.29	82.29	82.29
AHDB9849	88.54	90.63	90.63	90.63	90.63
AHDB9807	76.04	76.04	76.04	76.04	76.04
AHDB9850	89.58	89.58	89.58	89.58	90.63
AHDB9847	85.42	85.42	85.42	86.46	86.46
AHDB9763	83.33	85.42	85.42	85.42	85.42
AHDB9848	82.29	83.33	83.33	84.38	84.38
	Significantly different from the untreated inoculated control (p<0.05)				
	Not significantly different from the untreated inoculated control (p>0.05)				

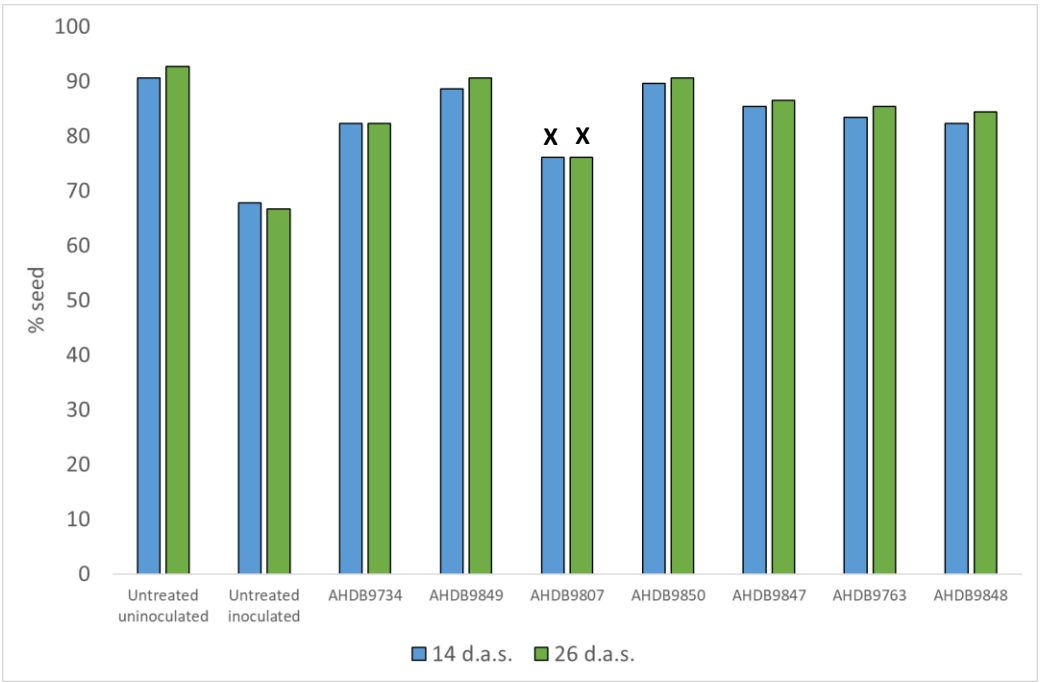


Figure 1. Effect of fungicides, bioprotectants and a liquid microbial fertilizer on mean seedling emergence (%) for each of five assessment dates.
X: Not significantly greater than the untreated inoculated control

F. culmorum incidence

- Fusarium incidence was characterized by the number of seedlings which collapsed after emergence due to infection, as well as the presence of Fusarium fungal growth on seedlings.
- Artificial inoculation of the growing media with *F. culmorum* was successful as the incidence of Fusarium significantly lower ($p<0.001$) in uninoculated untreated seedlings (1.0%) compared with inoculated untreated seedlings (29.17%) at 26 d.a.s. (Table 4).
- Low levels of Fusarium developed on seedlings in the uninoculated growing media, likely due to low background levels of Fusarium on the seed lot used.
- Three treatments, AHDB9850, AHDB9847 and AHDB9848 resulted in statistically significant control, reducing Fusarium incidence at all assessment dates, with all comparable to the uninoculated control ($p<0.001$, **Error! Reference source not found.**)
- AHDB9850 provided the best disease control, with no seedlings symptomatic of Fusarium until the final assessment (3.1%, 26 d.a.s.).
- AHDBYYY and AHDB9807 resulted in no disease control compared with the inoculated control.
- AHDB9849 resulted in some control compared with the inoculated control (3.1% vs. 15.6%, $p<0.001$) at 14 d.a.s., however no significant reductions were recorded at the other assessments.
- A significantly higher disease incidence was recorded in AHDB9763 compared with the inoculated control at every assessment date ($p<0.001$).

Table 4. Effect of fungicides, bioprotectants on mean Fusarium incidence (%) for each of five assessment dates.

Treatment	% Fusarium incidence				
	21/07/21 14 d.a.s.	23/07/21 16 d.a.s.	26/07/21 19 d.a.s.	30/07/21 23 d.a.s.	02/08/21 26 d.a.s.
Untreated uninoculated	2.08	2.08	1.04	1.04	1.04
Untreated inoculated	15.63	18.75	20.83	26.04	29.17
AHDB9734	9.38	17.71	18.75	20.83	30.21
AHDB9849	3.13	7.29	11.46	18.75	27.08
AHDB9807	10.42	15.63	21.88	26.04	33.33
AHDB9850	0.00	0.00	0.00	0.00	3.13
AHDB9847	3.13	3.13	7.29	11.46	13.54
AHDB9763	28.13	31.25	41.67	42.71	45.83
AHDB9848	0.00	2.08	3.13	5.21	8.33
	Significantly lower than the untreated inoculated control ($p<0.05$)				
	Significantly greater than the untreated inoculated control ($p<0.05$)				
	Not significantly different from the untreated inoculated control ($p>0.05$)				

Percentage reduction in Fusarium incidence compared with the untreated inoculated control, at each assessment date is shown in Table 5 and further highlight product efficacy at each assessment.

Table 5. Percentage reduction in mean Fusarium incidence treatments compared with the untreated control (Abbott's formula).

Treatment	% Fusarium incidence				
	21/07/21 14 d.a.s.	23/07/21 16 d.a.s.	26/07/21 19 d.a.s.	30/07/21 23 d.a.s.	02/08/21 26 d.a.s.
AHDBYYY	43.2	3.5	11.5	20.7	-3.5
AHDB9489	94.7	80.0	45.4	29.4	6.8
AHDB9807	32.1	16.1	-6.2	-1.2	-14.7
AHDB9850	100.0	100.0	100.0	100.0	94.7
AHDB9847	84.5	87.0	64.4	55.6	54.0
AHDB9763	-81.5	-66.1	-104.3	-64.6	-57.1
AHDB9848	100.0	97.1	92.4	85.7	72.1
	Significantly lower than the untreated inoculated control ($p<0.05$)				
	Significantly greater than the untreated inoculated control ($p<0.05$)				

	Not significantly different from the untreated inoculated control (p>0.05)

Conclusions

Germination:

- No treatment reduced overall seed germination rates compared with the untreated control in germination tests carried out one week and five weeks after seed treatment application.
- The number of weak seedlings was significantly lower in seed treated with AHDB9849, AHDB9807 and AHDB9848 during the early germination test.
- No difference in seedling quality were reported between the untreated and treated seedlings at the late germination test.

Disease control efficacy

- Low levels of naturally occurring Fusarium were present in the seed batch used for this work.
- Artificial inoculation of the soil with *F. culmorum* was successful.
- All treatments apart from the fungicide AHDB9807 significantly increased seedling emergence in soil artificially inoculated with *F. culmorum* to levels comparable to the uninoculated, untreated control.
- The three fungicides AHDB9850, AHDB9848 and AHDB9847 performed best, significantly reducing ($p<0.001$) visible Fusarium incidence by 95%, 72% and 54% respectively 26 days after sowing in soil artificially inoculated with *F. culmorum*.
- The bioprotectants AHDB9734 and AHDB9849 did not reduce incidence of Fusarium on emerging leek seedlings.
- AHDB9763 increased Fusarium incidence by 57% compared with the inoculated untreated control 26 days after sowing.

Overall

- All treatments applied at the concentrations tested had no negative impact on germination rates or seedling quality characteristics and are considered to be crop safe on leek one week, and five weeks after application.
- The three fungicides AHDB9850, AHDB9848 and AHDB9847 are the most promising products to take forward and resulted in the best control against Fusarium in leek.
- The bioprotectants AHDB9734 and AHDB9849 increased seedling emergence but did not reduce disease incidence. However, these products could potentially be a valuable component of IPDM programmes when used under a lower disease pressure. Further work is required to confirm this.
- AHDB9763 is not suitable to be taken forward at the treatment rate tested.

Take home message:

In a comparison of treatments encouraging results were obtained with the fungicides AHDB9850, and to a lesser extent AHDB9848 and AHDB9847.

Full report

Summary

The fungal pathogens *F. culmorum* and *Fusarium oxysporum* f. sp*. cepae* are some of the pathogens responsible for causing damping off in leek. This disease causes emerging seedlings to collapse, often submerged in a mass of white fungal growth. Fusarium is one of the most destructive diseases of field-grown leek crops, with inoculum overwintering on crop debris in the soil. Survival in this manner enables Fusarium to infect newly developing seedlings.

Seed treatment remains an important component of disease management against Fusarium, however effective control is threatened by the recent loss of thiram, the standard seed treatment which is applied as a warm water soak. Non-chemical alternatives to thiram would be of major benefit to both organic and conventional growers, given continued consumer and retailer pressure for a reduction in the use of chemical fungicide products.

This study investigated potential new products applied as seed treatments for control of damping off for use in both organic and conventionally grown leek. This trial investigated seven seed treatments, four conventional fungicides, two bioprotectants and a liquid microbial fertilizer for control of *F. culmorum* in artificially inoculated growing media. The trial, conducted under controlled environment conditions identified potential new seed treatments for leek fusarium that could provide an alternative to thiram, for both conventional and organic celery production.

Objectives

1. To evaluate fungicides, bioprotectants and a liquid microbial fertilizer as potential seed treatments for efficacy against Fusarium damping-off in leek (*F. culmorum*).
2. To assess their crop safety in leek.

Trial conduct

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

Relevant EPPO guideline(s)		**Variation from EPPO**
PP 1/152(3)	Design and analysis of efficacy evaluation trials	None
PP 1/135(4)	Phytotoxicity assessment	None
PP 1/181(4)	Conduct and reporting of efficacy evaluation trials including good experimental practice	None
PP 1/214(3)	Principles of acceptable efficacy	None
PP 1/125(4)	Seed treatments against seedling diseases (trials under controlled conditions)	None

Test site

Item	**Details**
Location address	Boxworth, Cambridge CB23 4NN
Crop	Leek
Cultivar	Musselborough
Growing media	John Innes no. 1 (seed compost)
Agronomic practice	N/A
Prior history of site	N/A

Trial design

Germination tests

Two germination tests were established in the controlled environment (CE) cabinets at ADAS Boxworth to test the germination rates of untreated seed and seed treated with the test products. Germination was assessed one week after seed treatment was applied and again after the seed had been stored for five weeks under refrigerated conditions (4-8ºC, under darkness). Germination experiments were carried out at 20ºC, under 18:6 light:dark conditions. Fresh weights per plot were also recorded at the last assessment.

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:	50
Number of seeds per treatment:	200

Efficacy trial

Growing media was artificially inoculated with a known pathogenic isolate of *F. culmorum* and mixed to a final spore concentration of 10^4 spores per g (confirmed via serial dilutions). Twenty-four treated seeds were sown 2 cm deep in an 8x3 grid pattern. Seedling emergence and incidence (presence or absence) of Fusarium symptoms were recorded.

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

Treatment details

AHDB Code	Active substance	Product name or manufacturers code	Formulation batch number	Content of active substance in product	Formulation type[1]	Product type
N/A	N/A	Untreated	N/A	N/A	N/A	N/A
AHDB9734	N/D	N/D	N/K	N/D	Flowable concentrate	Bioprotectant
AHDB9849	N/D	N/D	0015463260	N/D	Flowable concentrate	Bioprotectant
AHDB9807	N/D	N/D	H15507021	N/D	Flowable concentrate	Fungicide
AHDB9850	N/D	N/D	PE-121615M08D015	N/D	Flowable concentrate	Fungicide
AHDB9847	N/D	N/D	EM4L022326	N/D	Flowable concentrate	Fungicide
AHDB9763*	N/D	N/D	N/K	N/D	Flowable concentrate	Microbial fertilizer
AHDB9848	N/D	N/D	2018 - 005011	N/D	Flowable concentrate	Fungicide

*included due to limited availability of candidate active substances/plant production products at the start of the trial.

Methods, assessment and records

Approximately 1 kg of untreated leek seed of a susceptible variety, Musselborough (1000 seed weight – 3.30 g) was obtained from a commercial supplier. All seeds were surface sterilized in the pathology laboratory at ADAS Boxworth (see below) before being sent to Elsoms Seeds Ltd. to be coated with the different product treatments.

Application details

Surface sterilization
Seeds were soaked in a 1% sodium hypochlorite solution for 30 seconds, followed by three 1 minute rinses in sterile distilled water (SDW) and then dried in a laminar flow hood. Seed was stored in the dark under cool (ca. 5^{o}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. 50g of seeds was sampled for each treatment, including controls. Seed was stored in paper bags, stored under dark, cold (ca. 4-8^{o}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^{o}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 active substance (ml or g a.s./ha)**	**Rate of product (l or kg/ha)**	**Application code**
1	Untreated	N/A	N/A	A
2	AHDB9734	N/A, seed treatment	200 ml per 100 kg	A
3	AHDB9849	N/A, seed treatment	160 ml per 100 kg seed	A
4	AHDB9807	N/A, seed treatment	200 ml per 100 kg seed	A
5	AHDB9850	N/A, seed treatment	100 ml per 100 kg seed	A
6	AHDB9847	N/A, seed treatment	100 ml per 100 kg seed	A
7	AHDB9763	N/A, seed treatment	500 ml per 100 kg seed	A
8	AHDB9848	N/A, seed treatment	1000 ml per 100 kg seed	A

Untreated levels of pests/pathogens at application and through the assessment period: seed treatment efficacy trial

Common name	Scientific Name	EPPO Code	Infection level pre-application	Infection level at start of assessment period	Infection level at end of assessment period
Leek Fusarium	*Fusarium culmorum*	FUSACU	Present	0% incidence	29% Fusarium incidence (inoculated untreated plants)

Assessment details

Phytotoxicity

Germination tests were set up one week and five weeks after treatment. Phytotoxic effects of the seed treatments on germination were assessed on damp filter paper placed in sealed plastic germination boxes.

Seed germination on filter paper

Each treated seed batch was subsampled for 50 seeds. Filter papers moistened with SDW were used to line plastic trays and these seeds were sown in a 10 x 5 grid. Trays were covered with lids to prevent moisture loss and incubated in controlled environment cabinet at 20°C with a 16:8 hour light:dark cycle for 28 days. Boxes were checked every 2-3 days to ensure the filter paper remained moist. There were four replicate boxes per treatment and the counts were performed every 7 days to identify any treatment effects on growth.

In addition to germination counts, seedling quality was also assessed after 28 days using the following categories:

- Germinated with normal development: cotyledons at least 50% emerged with no damage to terminal bud, roots > 1.0 cm.
- Germinated with weak growth and roots 0.5 – 1.0 cm.
- Germinated with abnormal growth and roots <0.5 cm.
- Ungerminated viable seed: seeds which remain firm and apparently viable at the end of the test.
- Ungerminated dead seed: seeds which at the end of the test period were either decayed, mouldy or soft or have not produced any seedling or part of a seedling.

Total plot fresh weight (g) for each germination test were also recorded at the final destructive assessment

Seed germination after storage

After establishment of the first germination test, all treated seed was stored in paper bags, placed into a box containing sachets of silica gel and refrigerated for 5 weeks in the ADAS Boxworth Pathology laboratory refrigerator. After four weeks, seed germination tests were set up as again described previously.

Determination of optimal *F. culmorum* inoculum rate

Inoculum preparation

Culture preparation
An isolate of *F. culmorum* (provided by Dr John Clarkson, University of Warwick) was used in this study. Cultures were 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 7 days or when the mycelial growth had just reached the edge of the plate i.e. actively growing.

Substrate preparation
A 1:10 ratio of milled wheat bran: John Innes no. 1 growing media was prepared, 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
Five 5 mm PDA discs of actively growing *F. culmorum* were taken and added to 600 g of preprepared substrate in sterilised conical flasks sealed with aluminium foil. Flasks were placed in an incubator at 20°C on an 18:6 light:dark cycle and mixed by shaking 2-3 times per

week. Before use the mixture was sieved through a 4 mm sieve to create a free-flowing, homogenous mixture. The contents of the conical flasks were combined and mixed, before passing through a 4 mm sieve to produce a uniform substrate. Spore concentrations were calculated by serial dilution.

Growth media preparation
John Innes No. 1 growing media was autoclaved twice for 2 hours at 121°C to sterilize before use and 400 g dispensed into plastic trays. Some sterile media was reserved for use in the uninoculated controls, but the rest was inoculated by mixing the inoculum stock with John Innes No. 1 growth media to the desired ratio. Different mixtures were prepared to give 10^4 10^5, 10^6 and 10^7 inoculum concentrations. 35 leek seeds were sown into the four different concentrations of *F. culmorum* inoculated growth media and incubated at 20°C with a 16:8 hour light:dark cycle for 21 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 resulted in a reduction of germination of 50-90% in the inoculated control compared to the uninoculated control.

Evaluation of seed treatment efficacy

For the disease control efficacy trial, an inoculum concentration of 10^4 spores per g was used and made by uniformly mixing the stock inoculum with the autoclaved John Innes No. 1 growing media. This concentration was confirmed by serial dilution.

Seed sowing and incubation
400 g of John Innes No. 1 growing media (uninoculated or inoculated with *F. culmorum*) was put into each seed tray (17.5 x 11.5 cm) which 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 CE cabinets at 20°C at 80% relatively humidity under an 18:6 light:dark cycle. Once emergence of seedlings had begun, the lids were removed.

At each assessment point the following were assessed as follows:

- Number of emerged seedlings.
- Number of seedlings failing to emerge.
- Disease incidence (percent of seedling damping off, or presence of visible F. culmorum growth on seedlings).

Assessment schedule
Seed were sown on 07/07/2021 and the trial was assessed every 2-4 days after the start of emergence.

Assessment no.	Assessment date	Seedling emergence in the uninoculated control (%)	Seedling emergence in the inoculated control (%)
1	21/07/21	90.6	66.7
2	23/07/21	90.6	66.7
3	26/07/21	91.7	66.7
4	30/07/21	91.7	66.7
5	02/08/21	92.7	66.7

Statistical analysis
The germination tests were laid out in a restricted randomised design, whilst the efficacy trial was laid out as a randomised complete block design. Statistical analysis was carried out by the ADAS statistician using ANOVA with a Duncan's Multiple Range Test in Genstat 18. To assess for differences between treatments compared to the untreated control, germination, disease incidence, 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 Table 10. Abbott's formula was also used to calculate the percentage change for each treatment relative to the control.

Results

Phytotoxicity: Germination tests

1. Germination immediately after treatment

No differences in the overall number of germinated seeds were recorded between treated and untreated seeds at the four assessments (p=0.838) with 92.0% of the untreated seed germinating after 28 days (Table 6).

Assessments showed no significant differences (p=0.480) in the number of healthy seeds (those germinated with normal development) at the 28 day destructive assessment (Table 6, Figure 3). However, at this time significantly fewer (p=0.005) seedlings classes as weak were recorded in seed treated with AHDB9849 (2.5%), AHDB9807 (1.0%) and AHDB9848 (1.5%) compared with the untreated control (6.5%). No differences in the number of abnormal seed were present between treatments (p=0.771).

For ungerminated seeds no differences between the number of viable (hard) seed (p=0.052) and the number of dead (soft) seed (p=0.385) were recorded between the untreated control and any of the seed which received treatment.

Table 6. Effect of treatments on leek seed germination and the quality of seedling development 28 days after sowing – first germination trial, 02 August 2021

Treatment	Overall germination (%)	Mean number seeds germinated (%)			Mean number of seeds not germinated (%)	
		Normal	Weak	Abnormal	Viable seed	Dead seed
Untreated	92.0	82.5	6.5	3.0	5.0	3.0
AHDB9734	90.5	81.0	7.5	2.0	2.0	7.5
AHDB9849	89.0	84.0	2.5	2.5	1.0	10.0
AHDB9807	89.0	83.5	1.0	4.5	4.0	7.0
AHDB9850	91.0	85.5	3.5	2.0	2.5	6.5
AHDB9847	91.5	84.5	4.0	3.0	2.5	6.0
AHDB9763	93.0	89.0	3.0	1.0	1.0	6.0
AHDB9848	90.5	87.5	1.5	1.5	2.5	7.0
p value	0.838	0.480	0.005	0.771	0.052	0.385
d.f.	21	21	21	21	21	21
s.e.d.	2.524	3.747	1.586	2.027	1.240	2.589
l.s.d.	5.250	7.792	3.298	4.216	2.580	5.384
	Significantly different from the untreated control (p<0.05)					
	Not significantly different from the untreated control (p>0.05)					

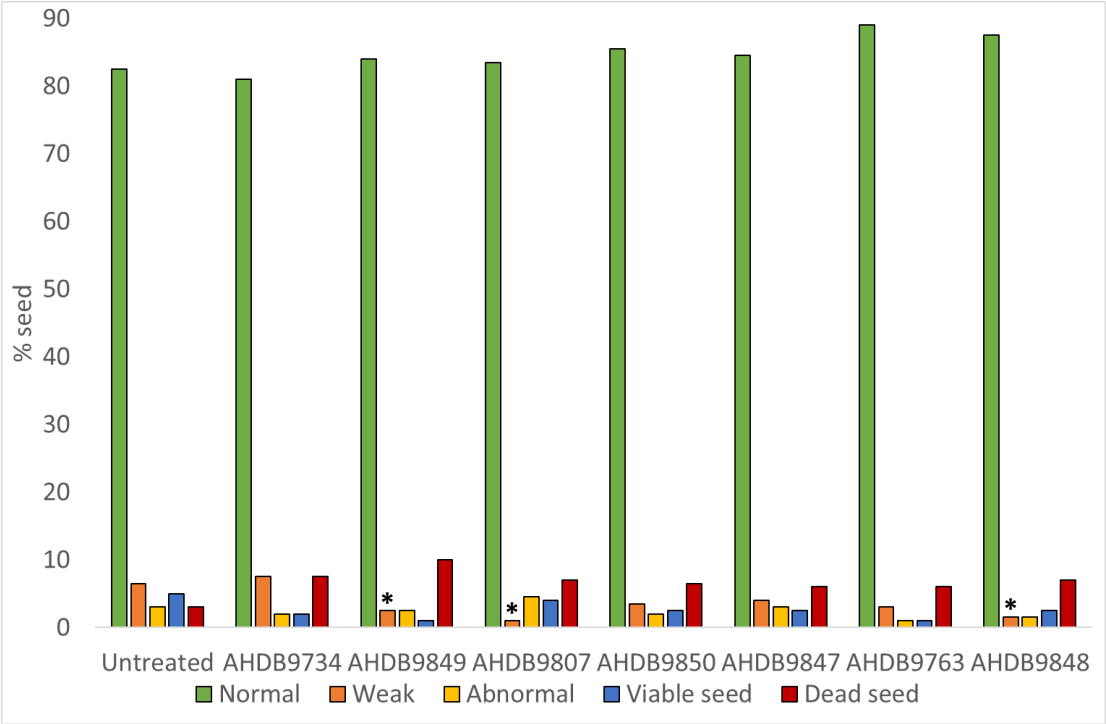


Figure 2. Effect of fungicides, bioprotectants and a liquid microbial fertilizer on leek seed germination and the quality of seedling development 28 days after sowing expressed as the percentage total of seeds assessed – early germination trial, 02 August 2021
Germinated seed: normal, weak or abnormal.
Ungerminated seed: viable (alive), or dead (soft/rotten).
* Significantly lower than the untreated control

Seed was stored for 1 month in the ADAS Boxworth Pathology laboratory fridge to determine any impacts storage had on the rate of seed germination and quality characteristics of the developing seedling quality (Table 7, Figure 4).

No treatment significantly reduced the overall germination rate (p=0.528) compared with the untreated control after 28 days. Similarly, no treatment significantly reduced (p>0.05) the number of normal, weak or abnormal seedlings or the number of ungerminated seeds (viable or dead) compared with the untreated control.

Table 7. Effect of treatments on leek seed germination and the quality of seedling development at 28 days after sowing – second germination trial, 06 September 2021

Treatment	Overall germination (%)	Mean number seeds germinated (%)			Mean number of seeds not germinated (%)	
		Normal	Weak	Abnormal	Viable seed	Dead seed
Untreated	89.5	83.0	6.0	0.5	4.0	6.5
AHDB9734	91.5	86.0	5.0	0.5	2.5	6.0
AHDB9849	87.5	79.5	4.5	3.5	3.5	9.0
AHDB9807	87.0	80.0	4.5	2.5	5.5	7.5
AHDB9850	86.5	80.0	4.5	2.0	3.5	10.0
AHDB9847	89.5	85.0	4.0	0.5	4.5	6.0
AHDB9763	88.0	84.5	3.0	0.5	3.5	8.5
AHDB9848	87.0	73.0	10.0	4.0	6.0	7.0
p value	0.528	0.064	0.283	0.107	0.749	0.663
d.f.	21	21	21	21	21	21
s.e.d.	2.557	3.914	2.594	1.472	2.113	2.472
l.s.d.	5.318	9.140	5.395	3.061	4.394	5.141
	Significantly different from the untreated control (p<0.05)					
	Not significantly different from the untreated control (p>0.05)					

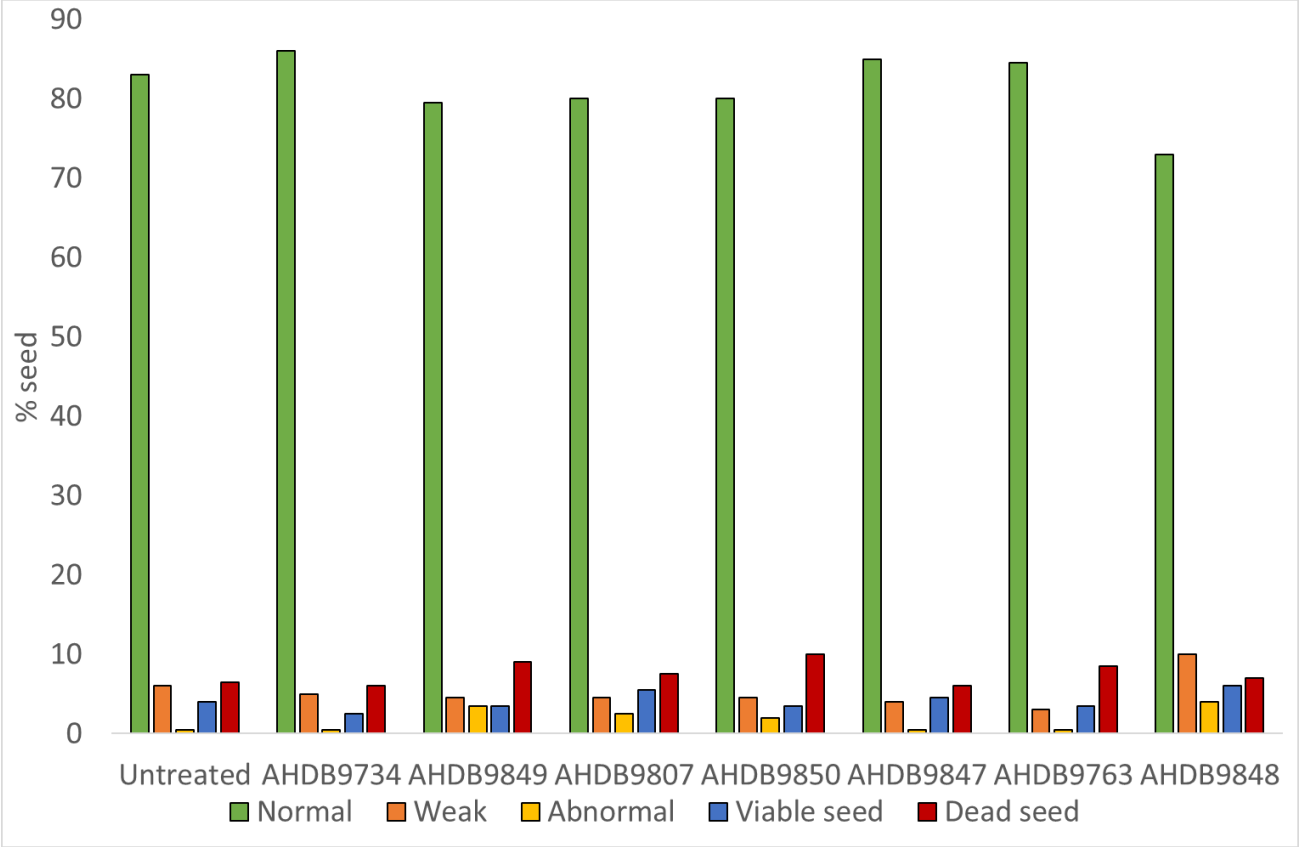


Figure 3. Effect of fungicides, bioprotectants and a liquid microbial fertilizer on leek seed germination and the quality of seedling development 28 days after sowing expressed as the percentage total of seeds assessed – 06 September 2021

Disease control efficacy

The disease control efficacy of the seed treatments was established using seedling emergence and counts of visible Fusarium incidence. Assessments began 14 days after sowing (d.a.s.) shortly after seedling emergence began. An untreated uninoculated control was run alongside an untreated inoculated control to confirm the pathogenicity of the *F. culmorum* isolate and to compare treatment effects.

1. Seedling Emergence

At the initial assessment (14 d.a.s.) most seedlings from seed that was able to germinate had already emerged. At the final assessment (26 d.a.s.) seedling emergence was significantly reduced from 92.7% in the uninoculated untreated control to 66.7% in the inoculated untreated control (p=0.001, Table 8, Figure 5.). Seeds were sown in a 3 x 8 grid with the aim of retrieving unemerged seedlings to identify the reason behind this. It was not possible to locate every unemerged seed and this process was abandoned. Interestingly, the emergence rate of untreated, uninoculated seedlings at 26 d.a.s. (92%) was greater than the germination rate in the (uninoculated) germination trials (83% in both).

All treatments, apart from AHDB9807 increased seedling emergence compared with the untreated inoculated control at every assessment date. At the final assessment (26 d.a.s.), seedling emergence was greatest in seed treated with AHDB9849 (90.6%), AHDB9850 (90.6%) and AHDB9847 (86.5%) compared with the untreated inoculated (66.7%) and AHDB9807 (76.0%, p=0.001). No significant differences were recorded between AHDB9734, AHDB9849, AHDB9850, AHDB9847, AHDB9763, AHDB9848 and the uninoculated untreated control (l.s.d. 11.01).

Table 8. Effect of treatments on mean seedling emergence (%) for each of five assessment dates.

Treatment	% emergence				
	21/07/21 14 d.a.s.	23/07/21 16 d.a.s.	26/07/21 19 d.a.s.	30/07/21 23 d.a.s.	02/08/21 26 d.a.s.
Untreated uninoculated	90.63	90.63	91.67	91.67	92.71
Untreated inoculated	67.71	66.67	66.67	66.67	66.67
AHDB9734	82.29	82.29	82.29	82.29	82.29
AHDB9849	88.54	90.63	90.63	90.63	90.63
AHDB9807	76.04	76.04	76.04	76.04	76.04
AHDB9850	89.58	89.58	89.58	89.58	90.63
AHDB9847	85.42	85.42	85.42	86.46	86.46
AHDB9763	83.33	85.42	85.42	85.42	85.42
AHDB9848	82.29	83.33	83.33	84.38	84.38
p value	0.007	0.003	0.002	0.002	0.001
d.f.	24	24	24	24	24
s.e.d.	5.43	5.45	5.34	5.27	5.33
l.s.d.	11.20	11.24	11.03	10.88	11.01
	Significantly different from the untreated inoculated control (p<0.05)				
	Not significantly different from the untreated inoculated control (p>0.05)				

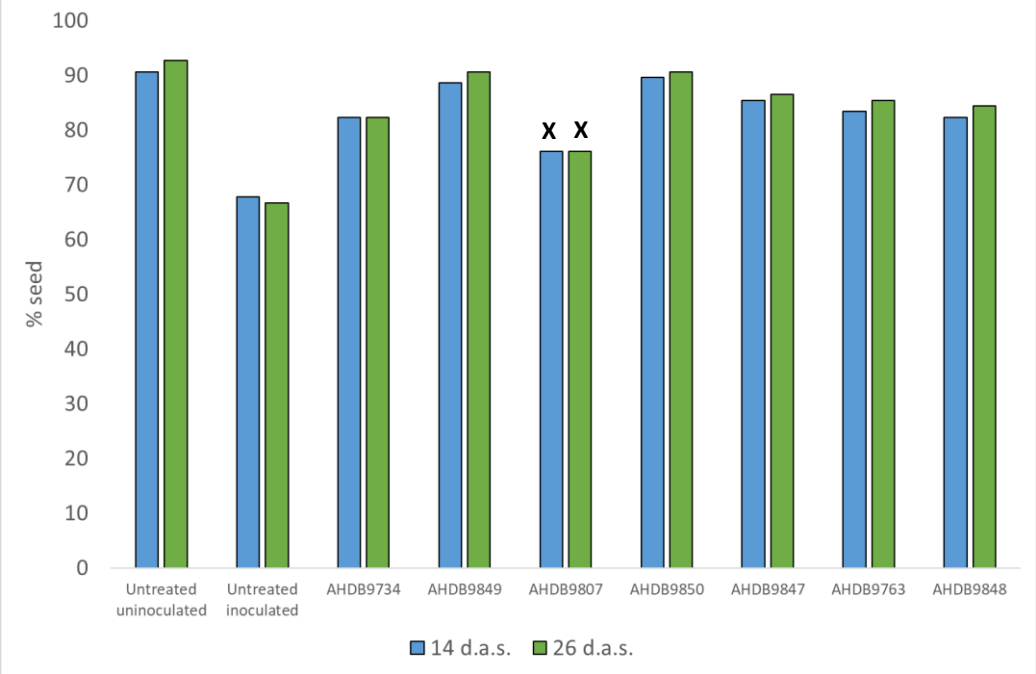


Figure 4. Effect of fungicides, bioprotectants and a liquid microbial fertilizer on mean seedling emergence (%) for each of five assessment dates.
X: Not significantly greater than the untreated inoculated control

2. Fusarium incidence

Fusarium incidence was characterized by the number of seedlings which collapsed after emergence due to infection, as well as the presence of Fusarium fungal growth on seedlings. Artificial inoculation of the growing media was successful using the rate identified in the preliminary work. At all assessments, the incidence of Fusarium was significantly lower ($p<0.001$) in untreated seedlings grown in uninoculated growing media compared with untreated seedlings grown in the inoculated growing media.

Unexpectedly, low levels of Fusarium developed on seedlings in the uninoculated growing media. One seed in the early germination test (untreated) was confirmed to be infected with Fusarium and it is likely that the pathogen was present in the seed batch at low levels. This level of natural infection was low and is not anticipated to have impacted the validity of the results from this work.

Generally, Fusarium levels increased over time with incidence levels in the untreated inoculated seedlings 15.6% at 14 d.a.s. and 29.2% by 26 d.a.s. (Table 9, Figure 6). Fusarium levels in the untreated uninoculated remained low. Of the seven seed treatments tested, AHDBYYY and AHDB9807 provided no disease control with the presence of Fusarium comparable to the inoculated control. At 14 d.a.s. AHDB9849 resulted in some control compared with the inoculated control (3.1% vs. 15.6%, $p<0.001$), however no significant reductions were recorded at the other assessments.

Three treatments, AHDB9850, AHDB9847 and AHDB9848 resulted in statistically significant control, reducing Fusarium incidence at all assessment dates, with all comparable to the untreated uninoculated control ($p<0.001$). AHDB9850 provided the best disease control, with no seedlings symptomatic of Fusarium until the final assessment (3.1%, 26 d.a.s.). Fusarium symptoms developed at the second assessment in AHDB9848 treated seeds (2.1%, 16 d.a.s.), increasingly slowly to 8.3% by 26 d.a.s. A higher disease incidence was recorded for AHDB9847 with symptoms present at the fist assessment (3.1%, 14 d.a.s.), increasing to 13.5% at 26 d.a.s.

Seedlings developing from seed treated with AHDBZZZ resulted in a statistically significant increase ($p<0.001$) in Fusarium incidence compared with the untreated inoculated control at the first assessment (28.1% vs. 15.6% respectively). This continued at all further assessments with an incidence of 45.8% in AHDBZZZ treated seed, compared with 29.2% in the untreated inoculated seed. The reason behind this is unknown.

Table 9. Effect of treatments on mean Fusarium incidence (%) for each of five assessment dates.

Treatment	% Fusarium incidence				
	21/07/21 14 d.a.s.	23/07/21 16 d.a.s.	26/07/21 19 d.a.s.	30/07/21 23 d.a.s.	02/08/21 26 d.a.s.
Untreated uninoculated	2.08	2.08	1.04	1.04	1.04
Untreated inoculated	15.63	18.75	20.83	26.04	29.17
AHDB9734	9.38	17.71	18.75	20.83	30.21
AHDB9849	3.13	7.29	11.46	18.75	27.08
AHDB9807	10.42	15.63	21.88	26.04	33.33
AHDB9850	0.00	0.00	0.00	0.00	3.13
AHDB9847	3.13	3.13	7.29	11.46	13.54
AHDB9763	28.13	31.25	41.67	42.71	45.83
AHDB9848	0.00	2.08	3.13	5.21	8.33
p value	<0.001	<0.001	<0.001	<0.001	<0.001
d.f.	24	24	24	24	24
s.e.d.	3.944	5.63	6.23	5.91	5.42
l.s.d.	8.139	11.61	12.86	12.19	11.18
	Significantly lower than the untreated inoculated control ($p<0.05$)				
	Significantly greater than the untreated inoculated control ($p<0.05$)				
	Not significantly different from the untreated inoculated control ($p>0.05$)				

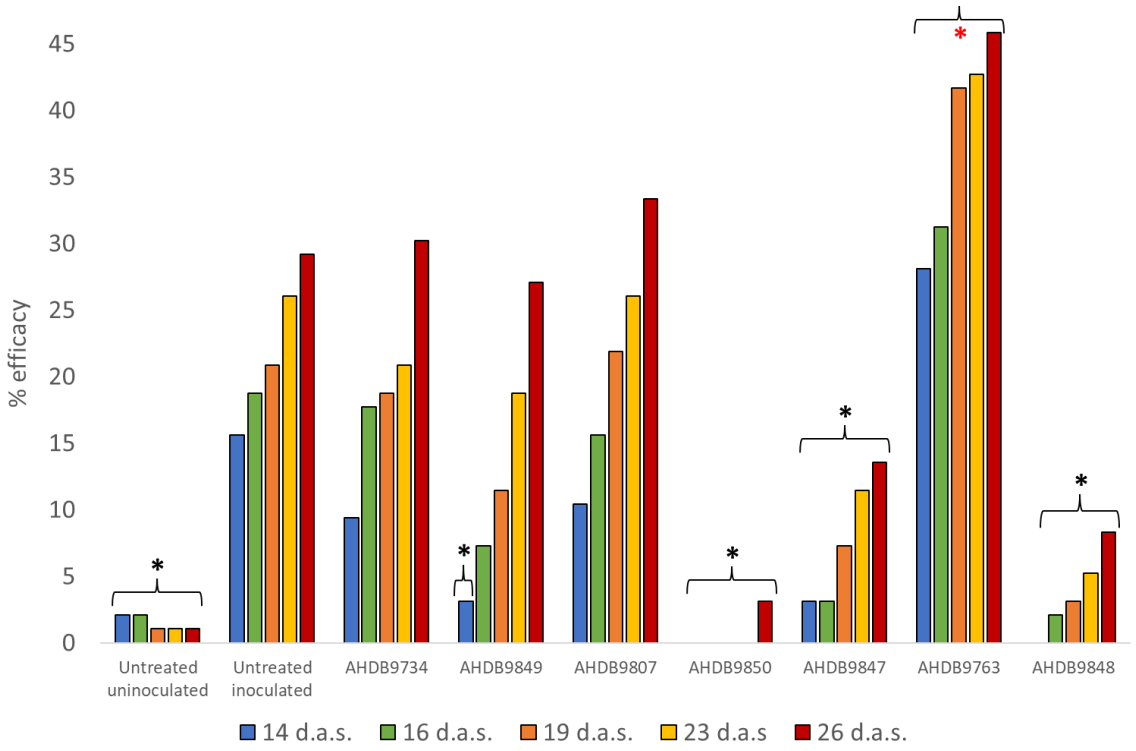


Figure 5. Effect of fungicides, bioprotectants and a liquid microbial fertilizer on mean Fusarium incidence (%) for each of five assessment dates.

*Significantly lower than the untreated inoculated control

*Significantly greater than the untreated inoculated control

Percentage reduction in Fusarium incidence compared with the untreated inoculated control, at each assessment date are shown in Table 10 and further highlight product efficacy at each assessment.

Table 10. Percentage reduction in mean Fusarium incidence for treatments compared with the untreated control (Abbott's formula).

Treatment	% Fusarium incidence				
	21/07/21 14 d.a.s.	23/07/21 16 d.a.s.	26/07/21 19 d.a.s.	30/07/21 23 d.a.s.	02/08/21 26 d.a.s.
AHDB9734	43.2	3.5	11.5	20.7	-3.5
AHDB9489	94.7	80.0	45.4	29.4	6.8
AHDB9807	32.1	16.1	-6.2	-1.2	-14.7
AHDB9850	100.0	100.0	100.0	100.0	94.7
AHDB9847	84.5	87.0	64.4	55.6	54.0
AHDB9763	-81.5	-66.1	-104.3	-64.6	-57.1
AHDB9848	100.0	97.1	92.4	85.7	72.1
	Significantly lower than the untreated inoculated control (p<0.05)				
	Significantly greater than the untreated inoculated control (p<0.05)				
	Not significantly different from the untreated inoculated control (p>0.05)				

Discussion

Two germination tests, one seven days after treatment application and another after five weeks of storage, evaluated the impact of seed treatments on both the germination rate and the quality of emerging leek seedlings. A pathogenicity assay using growing media artificially

inoculated with *F. culmorum* was then used to assess the efficacy of seven seed treatment products. Levels of leek Fusarium were established at sufficient levels to enable significant differences between test treatments and the untreated control to be observed.

In addition to the four conventional fungicide treatments, two *Bacillus* based bioprotectants and a liquid microbial fertilizer were tested. Bioprotectants and some microbial fertilisers have been demonstrated to have antifungal properties, have a low environmental impact and leave no residues of concern on treated crops, thus justifying their investigation in this study.

There was no negative impact of any of the treatments on leek seed germination or emergence. The number of 'normal' germinated seedlings which developed, compared with the untreated control in the first and second germination tests, was also the same. In the first germination test only, the number of 'weak' germinated seedlings was reduced in three treatments, AHDB9849, AHDB9807 and AHDB848, compared with the control but this did not correspond with a significant increase in the number of abnormal seedlings recorded. The germination rates were slightly lower in the second germination. Experimental conditions were identical, so this difference is most likely be attributed to the age of the seed and some deterioration after storage. Overall, the results of the two germination tests are positive and suggest that all treatments used in this work are crop safe at the rates tested but using freshly treated seed with give marginally better germination rates.

All seed treatments apart from the fungicide AHDB9807 consistently increased seedling emergence compared with the untreated inoculated control, to levels comparable to the untreated uninoculated seed. This included the two bioprotectants and the liquid microbial fertilizer (AHDB9734, AHDB9849 and AHDB9763 respectively). These products therefore protected seed prior to emergence, or enhanced subsequent germination allowing them to emerge earlier. However, no differences in the date of emergence, seedling size, or seedling vigour were recorded at either the first or second germination tests.

Three of the four conventional fungicides, AHDB9850, AHDB9848 and AHDB9847 significantly reduced Fusarium incidence to similar levels to the untreated, uninoculated control. AHDB9850 performed best reducing Fusarium incidence by 95% at the final assessment, followed by AHDB9848 and AHDB9847 which reduced disease incidence by 72% and 54% respectively. The fungicide product AHDB9807 provided no disease control.

Although all the bioprotectants increased seedling emergence to counts comparable to the untreated uninoculated control, they provided no significant control in reducing the incidence of Fusarium damping off after emergence. These products should not however be discounted as this trial was carried out under high disease pressure in artificially inoculated soil that is unlikely to be replicated in the field. Under a lower inoculum load these products may give significant reductions in damping off and they could still be a valuable component of integrated pest and disease management (IPDM) programmes, especially for growers of organic leek. Further work under these field conditions would be required to confirm this.

Use of the liquid microbial fertilizer AHDB9763 significantly increased the incidence of Fusarium on emerged seedlings by 57% compared with the untreated inoculated control by the final assessment. The reason for this is unknown, but seedlings appeared to be more susceptible to infection after emergence. This product showed promise in significantly increasing seedling emergence compared with the untreated inoculated control. This effect could potentially be reduced if AHDB9763 was applied at a lower rate. Further studies may need to consider treatment dose/response using a range of concentrations to extrapolate effective seed treatment rates. At its current rate it is not recommended for use against Fusarium in leek.

In a comparison of treatments AHDB9850 was the most effective treatment in reducing Fusarium damping off in leek without affecting seed germination or vigour. However, comparable results were obtained with AHDB9848 and to a lesser extent AHDB9847, indicating their promise of these products as future seed treatment options for leek.

Conclusions

Germination:

- No treatment affected leek seed germination compared with the untreated control in tests carried out one week and five weeks after seed treatment application.
- The number of weak seedlings was significantly lower in seed treated with AHDB9849, AHDB9807 and AHDB9848 during the early germination test.
- No difference in seedling/root quality was evident between the untreated and treated seedlings at the second germination test.

Disease control efficacy

- Low levels of naturally occurring Fusarium were present in the seed batch used for this work.
- Artificial inoculation of the soil with *F. culmorum* was successful.
- All treatments apart from the conventional product AHDB9807 significantly increased seedling emergence in soil artificially inoculated with *F. culmorum* to levels comparable to the uninoculated, untreated control.
- The three fungicide products AHDB9850, AHDB9848 and AHDB9847 significantly reduced ($p<0.001$) Fusarium incidence by 95%, 72% and 54% respectively 26 days after sowing in soil artificially inoculated with *F. culmorum*.
- The bioprotectants AHDB9734 and AHDB9849 did not reduce incidence of Fusarium on emerging leek seedlings.
- AHDB9763 increased Fusarium incidence by 57% compared with the inoculated untreated control 26 days after sowing.

Overall

- All treatments applied at the concentrations tested had no negative impact on germination or seedling quality characteristics, and are considered to be crop safe on leek one week, and five weeks after application.
- The three fungicides AHDB9850, AHDB9848 and AHDB9847 are the most promising products to take forward and resulted in the best control against Fusarium in leek.
- The bioprotectants AHDB9734 and AHDB9849 increased seedling emergence, but did not reduce disease incidence. However, these products could be a valuable component of IPDM programmes when used under a lower disease pressure and further work is required to confirm this.
- AHDB9763 is not suitable to be taken forward at the treatment rate tested.

Acknowledgements

We would like to thank AHDB Horticulture and the participating crop protection companies for project funding. Thanks to John Clarkson (University of Warwick) for providing an isolate of *F. culmorum* and Elsoms Seeds Ltd. for providing seed treatment facilities.

Appendix

a. Crop diary – events related to growing crop

Crop	Cultivar	Treatment date
Leek	Musselborough	28 June 2021

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

Date	Event
Pretrial	
17/07/2020	*F. culmorum* arrived from Warwick University
18/08/2020 - 19/08/2020	Seed surface sterilised, air dried, subsampled and bagged. Seed stored in the dark ca. 5^{o}C
29/09/2020	*F. culmorum* optimal load tests established
06/10/2020	Optimal load assessments
05/07/2021	Seed treatment by Elsoms
Germination tests	
12/07/2021	Early germination - trial set-up
02/08/2021	Early germination – 28 day assessment
16/08/2021	Late germination trial set-up
06/09/2021	Late germination – 28 day assessment
Efficacy tests	
01/07/2021	Soil artificially inoculated with *F. culmorum* and placed in plastic trays
07/07/2021	Trial sown
21/07/2021	Assessment 1: Seedling emergence and disease incidence
23/07/2021	Assessment 2: Seedling emergence and disease incidence
26/07/2021	Assessment 3: Seedling emergence and disease incidence
30/07/2021	Assessment 4: Seedling emergence and disease incidence
02/08/2021	Assessment 5: Seedling emergence and disease incidence

c. Raw data from assessments

- Phytotoxicity - germination tests one week after seed treatment

Replicate	Plot	Assessment Date					
		Category of seedling/seed	Emerged seedling			Non emerged seed	
			Normal	Weak	Abnormal	Fresh	Dead
		Treatment Name					
1	101	Untreated	80	8	4	6	2
2	201	Untreated	84	4	6	2	4
3	301	Untreated	82	6	2	6	4
4	401	Untreated	84	8	0	6	2
1	102	AHDB9734	86	6	2	2	4
2	202	AHDB9734	82	10	2	0	6
3	302	AHDB9734	82	6	2	2	8
4	402	AHDB9734	74	8	2	4	12
1	103	AHDB9763	90	4	2	2	2
2	203	AHDB9763	88	2	0	0	10
3	303	AHDB9763	90	2	2	0	6
4	403	AHDB9763	88	4	0	2	6
1	104	AHDB9847	92	0	0	2	6
2	204	AHDB9847	84	8	0	4	4
3	304	AHDB9847	80	4	4	0	12
4	404	AHDB9847	82	4	8	4	2
1	105	AHDB9848	92	4	0	2	2
2	205	AHDB9848	90	0	2	4	4
3	305	AHDB9848	78	2	4	4	12
4	405	AHDB9848	90	0	0	0	10

Replicate	Plot	Assessment Date					
		Category of seedling/seed	Emerged seedling			Non emerged seed	
			Normal	Weak	Abnormal	Fresh	Dead
		Treatment Name					
1	106	AHDB9807	90	0	0	4	6
2	206	AHDB9807	84	2	0	6	8
3	306	AHDB9807	82	0	8	4	6
4	406	AHDB9807	78	2	10	2	8
1	107	AHDB9850	84	6	0	4	6
2	207	AHDB9850	82	6	4	2	6
3	307	AHDB9850	90	2	0	0	8
4	407	AHDB9850	86	0	4	4	6
1	108	AHDB9849	72	4	4	2	18
2	208	AHDB9849	86	0	2	2	10
3	308	AHDB9849	88	2	4	0	6
4	408	AHDB9849	90	4	0	0	6

- Phytotoxicity - germination tests five weeks after seed treatment

Replicate	Plot	Assessment Date					
		Category of seedling/seed	Emerged seedling			Non emerged seed	
			Normal	Weak	Abnormal	Fresh	Dead
		Treatment Name					
1	101	Untreated	80	6	0	2	12
2	201	Untreated	84	4	0	6	6
3	301	Untreated	78	10	2	4	6
4	401	Untreated	90	4	0	4	2
1	102	AHDB9734	88	2	2	4	4
2	202	AHDB9734	80	14	0	0	6
3	302	AHDB9734	92	0	0	4	4
4	402	AHDB9734	84	4	0	2	10
1	103	AHDB9763	84	2	2	4	8
2	203	AHDB9763	82	6	0	6	6
3	303	AHDB9763	90	2	0	2	6
4	403	AHDB9763	82	2	0	2	14
1	104	AHDB9847	84	4	0	6	6
2	204	AHDB9847	86	2	2	2	8
3	304	AHDB9847	86	6	0	2	6
4	404	AHDB9847	84	4	0	8	4
1	105	AHDB9848	82	4	2	6	6
2	205	AHDB9848	78	6	6	2	8
3	305	AHDB9848	72	14	2	4	8
4	405	AHDB9848	60	16	6	12	6
1	106	AHDB9807	86	2	0	8	4
2	206	AHDB9807	84	2	4	6	4
3	306	AHDB9807	74	6	2	6	12
4	406	AHDB9807	76	8	4	2	10
1	107	AHDB9850	86	4	0	2	8
2	207	AHDB9850	86	2	0	2	10
3	307	AHDB9850	78	4	2	2	14
4	407	AHDB9850	70	8	6	8	8
1	108	AHDB9849	82	4	8	2	4
2	208	AHDB9849	78	2	0	8	12
3	308	AHDB9849	84	4	2	4	6
4	408	AHDB9849	74	8	4	0	14

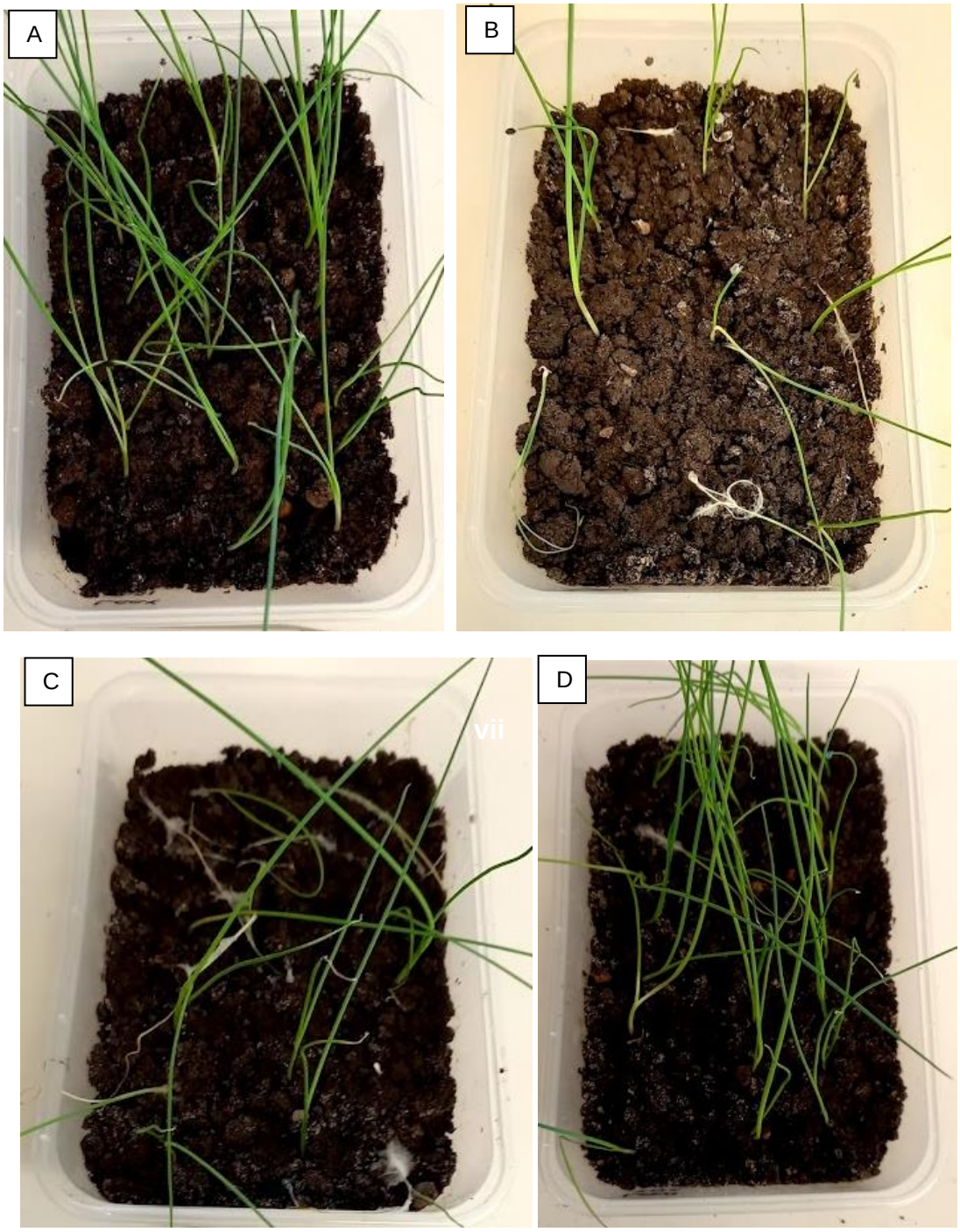
d. Efficacy trial - seed washing leaf inoculation plot data

Replicate	Plot	Assessment Date	21/07/2021		23/07/2021		26/07/2021		30/07/2021		02/08/2021	
		Day post inoculation	14		16		19		23		26	
		Assessment Type	Emerged (%)	Incidence (%)	Emerged (%)	Incidence (%)	Emerged (%)	Incidence (%)	Emerged (%)	Incidence (%)	Emerged (%)	Incidence (%)
1	101	AHDB9849	87.5	0.0	91.7	0.0	91.7	8.3	91.7	12.5	91.7	25.0
1	102	AHDB9763	70.8	20.8	83.3	12.5	83.3	20.8	83.3	20.8	83.3	25.0
1	103	AHDB9847	87.5	0.0	87.5	0.0	87.5	4.2	87.5	12.5	87.5	16.7
1	104	AHDB9807	79.2	12.5	79.2	20.8	79.2	29.2	79.2	29.2	79.2	29.2
1	105	Untreated inoculated	70.8	12.5	70.8	12.5	70.8	16.7	70.8	20.8	70.8	29.2
1	106	Untreated uninoculated	79.2	0.0	79.2	0.0	79.2	0.0	79.2	0.0	79.2	0.0
1	107	AHDB9848	83.3	0.0	87.5	0.0	87.5	4.2	87.5	4.2	87.5	4.2
1	108	AHDB9734	70.8	16.7	70.8	25.0	70.8	33.3	75.0	33.3	75.0	37.5
1	109	AHDB9850	87.5	0.0	87.5	0.0	87.5	0.0	87.5	0.0	87.5	0.0
2	201	Untreated uninoculated	91.7	0.0	91.7	0.0	91.7	0.0	95.8	0.0	95.8	0.0
2	202	AHDB9847	87.5	4.2	87.5	4.2	87.5	8.3	91.7	12.5	91.7	16.7
2	203	AHDB9734	87.5	12.5	87.5	16.7	87.5	20.8	87.5	20.8	87.5	33.3
2	204	AHDB9763	87.5	37.5	87.5	50.0	87.5	58.3	87.5	58.3	87.5	58.3
2	205	AHDB9848	75.0	0.0	75.0	8.3	75.0	8.3	79.2	12.5	79.2	12.5
2	206	AHDB9850	91.7	0.0	91.7	0.0	91.7	0.0	91.7	0.0	91.7	0.0
2	207	AHDB9849	91.7	12.5	91.7	20.8	91.7	20.8	91.7	33.3	91.7	33.3
2	208	Untreated inoculated	54.2	8.3	54.2	12.5	54.2	12.5	54.2	16.7	54.2	20.8
2	209	AHDB9807	83.3	12.5	83.3	20.8	83.3	29.2	83.3	29.2	83.3	29.2
3	301	Untreated uninoculated	95.8	0.0	95.8	0.0	95.8	0.0	95.8	0.0	100.0	0.0
3	302	AHDB9847	75.0	4.2	75.0	4.2	75.0	8.3	75.0	8.3	75.0	8.3
3	303	AHDB9850	91.7	0.0	91.7	0.0	91.7	0.0	91.7	0.0	95.8	4.2
3	304	Untreated inoculated	79.2	20.8	79.2	33.3	79.2	37.5	79.2	37.5	79.2	37.5
3	305	AHDB9763	91.7	37.5	91.7	41.7	91.7	54.2	91.7	54.2	91.7	62.5
3	306	AHDB9807	75.0	8.3	75.0	12.5	75.0	12.5	75.0	20.8	75.0	41.7
3	307	AHDB9734	87.5	4.2	87.5	12.5	87.5	12.5	87.5	12.5	87.5	20.8
3	308	AHDB9849	83.3	0.0	83.3	8.3	83.3	8.3	83.3	12.5	83.3	25.0
3	309	AHDB9848	95.8	0.0	95.8	0.0	95.8	0.0	95.8	0.0	95.8	8.3
4	401	AHDB9807	66.7	8.3	66.7	8.3	66.7	16.7	66.7	25.0	66.7	33.3
4	402	AHDB9763	83.3	16.7	79.2	20.8	79.2	33.3	79.2	37.5	79.2	37.5
4	403	AHDB9850	87.5	0.0	87.5	0.0	87.5	0.0	87.5	0.0	87.5	8.3
4	404	Untreated uninoculated	95.8	8.3	95.8	8.3	95.8	4.2	95.8	4.2	95.8	4.2
4	405	AHDB9848	75.0	0.0	75.0	0.0	75.0	0.0	75.0	4.2	75.0	8.3
4	406	AHDB9849	91.7	0.0	95.8	0.0	95.8	8.3	95.8	16.7	95.8	25.0
4	407	AHDB9847	91.7	4.2	91.7	4.2	91.7	8.3	91.7	12.5	91.7	12.5
4	408	Untreated inoculated	66.7	20.8	62.5	16.7	62.5	16.7	62.5	29.2	62.5	29.2
4	409	AHDB9734	83.3	4.2	83.3	16.7	83.3	8.3	79.2	16.7	79.2	29.2

e. Treatment images – 09 August 2021

A: Uninoculated untreated control
B: Inoculated untreated control
C: AHDB9850
D: AHDB9763

Note fluffy white Fusarium growth on seedlings in the inoculated untreated control, AHDB9850 and AHDB9763



f. 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