SCEPTREPLUS

# Final Trial Report

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|---|---|
| **Trial code:** | SP63a |
| **Title:** | Evaluation of new seed treatments for control of soil-borne Pythium in leek |
| **Crop** | Field vegetables - leek |
| **Target** | Leek Pythium (*Pythium ultimum*), PYTHUL |
| **Lead researcher:** | Dave Kaye (ADAS) |
| **Organisation:** | RSK ADAS Ltd. |
| **Period:** | April 2020 - 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: 02/02/2021 Authors signature: D. Kaye.

# Grower Summary

## Introduction

The fungal plant pathogen *Pythium ultimum* causes damping off in leek. The disease affects the roots and is characterized by seed rotting, failed emergence or the death of seedlings soon after they emerge. More established plants, or those grown under a lower inoculum load, may be able to grow through and survive infection with Pythium; however their rate of growth is usually reduced.

Seed treatment is an important part of managing Pythium disease in leek. Until recently Thiram was the standard treatment used, applied to the seed as a warm water soak. With the withdrawal of Thiram, alternative seed treatments are required to minimize the threat from *P. ultimum*. 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 leek against *P. ultimum* as an alternative to Thiram.

## Methods

Efficacy and crop safety experiments were conducted with leek seed of the susceptible variety, Porvite. Seeds were treated with 6 different crop protection products by Elsom's Ltd. All seeds also 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 Seeds Ltd
    - 100 seeds/treatment.
    - Scored for germination at 28 days (germinated/ungerminated)
2. 12 weeks post treatment – conducted by ADAS Horticulture
    - 12 weeks represents medium-term commercial storage practices.
    - 100 seeds/treatment.
    - Scored for emergence at 7 and 15 days.
    - Scored for seedling quality (1-5 scale) at 15 days.

**Seed treatment efficacy for control of *P. ultimum***

To test the disease control efficacy of the seed treatments, they were challenged in a controlled experiment by sowing into growing medium inoculated with a pathogenic isolate of *P. ultimum*.

- Preliminary study was conducted to determine optimal *P. ultimum* inoculum concentration to use
- Main disease control efficacy trials
    - 4 replicates of 24 seeds.
    - Sown into 200 g of 10% *P. ultimum* inoculated growth medium, grown at 20°C for 28 days (16:18 light dark cycle, 80% RH).
    - Assessed at 5 time points up to 25 days.
    - Scored for germination (% of total) and vigour (% of uninoculated control) at every time point.
    - Final assessment: Scored for disease incidence (%), disease severity (%) and sum of fresh weight (g).

## Results

### Seed phytotoxicity and germination tests

### 1. Trial 1. 1 week post treatment – conducted by Elsoms Seeds Ltd

- Overall germination rate lower than expected in untreated leek seed (64%) (Table 1)
- Filmcoat green did not have an effect on overall germination rate (62%)
- AHDB9815, a bioprotectant product had the greatest impact on germination (48%)
- AHDB9941 and AHDB 9848 had least impact on germination (60% and 58% germination respectively)
- AHDB9797 and AHDB9849 both decreased germination to 53%.
- Germination rate of untreated seed was already low enough to not be commercially acceptable. Further reductions on this rate would also not be commercially acceptable.

**Table 1.** Effect of crop protection products on germination of leek seeds immediately after treatment.

| Treatment code | Treatment | Germination % final | % difference in germination* |
|---|---|---|---|
| 1 | Untreated | 64 | |
| 2 | Filmcoat green | 62 | -3 |
| 3 | AHDB9797 | 53 | -17 |
| 4 | AHDB9849 | 53 | -17 |
| 5 | AHDB9815 | 48 | -25 |
| 6 | AHDB9941 | 60 | -6 |
| 7 | AHDB9848 | 58 | -9 |
| 8 | AHDB9955 | 49 | -23 |

*Relative to untreated control

### 2. Trial 2. 12 weeks post treatment – conducted by ADAS Horticulture

**Germination**

- Similar results to Elsoms Seeds Ltd. early germination tests (Trial 1), but untreated seed germination was decreased (Trial 1: 62%, Trial 2: 56%)
- AHDB9797 reduced germination compared with early germination test at 15 d assessments (Trial 1: 53%, Trial 2: 14%)
- At 15d assessment, germination slightly increased compared with early test for AHDB9848 (Trial 1: 61%, Trial 2: 58%) and AHDB9955 (Trial 1: 54%, Trial 2: 49%)
- AHDB9849, AHDB9941 had comparable germination rates between trials 1 and 2.
- AHDB9815 (biological product) slight reduction in germination (Trial 1: 48%, Trial 2: 43%).

- AHDB9815 germination was slow compared with other treatments and untreated control. Only 18% of the final number of germinated seeds had emerged at 7d, compared with between 71 and 96% for all other treatments.

**Seedling quality**

- At 7d seedling quality generally high. Between 4-4.5 for all treatments except AHDB9941 (3.5).
- 3 treatments had improved quality at 15d; AHDB9849, AHDB9941, AHDB9848.
- At 15d AHDB9955 had slight quality reduction.
- Filmcoat green impacted root size with many below 1cm at 7d and 15d, although plants appeared vigorous.
- AHDB9941 and AHDB9849 had greatest seedling quality (score of 5 at 15 d).

**Table 2.** Effect of crop protection products on leek seed germination and quality at 7 and 15 days after sowing following storage for 12 weeks

| | 7 d | | | 15 d | | |
|---|---|---|---|---|---|---|
| **Treatment** | **Germination %** | **% difference in germination*** | **Seedling quality (1-5 index)** | **Germination %** | **% difference in germination*** | **Seedling quality (1-5 index)** |
| Untreated control** | 53 | | 4.5 | 56 | | 4.5 |
| AHDB9797 | 10 | -81 | 4 | 14 | -75 | 4 |
| AHDB9849 | 48 | -9 | 4.5 | 52 | -29 | 5 |
| AHDB9815 | 8 | -85 | 4 | 43 | -25 | 4 |
| AHDB9941 | 57 | 8 | 3.5 | 60 | 9 | 5 |
| AHDB9848 | 57 | 8 | 4 | 61 | 8 | 4.5 |
| AHDB9955 | 52 | 2 | 4.5 | 54 | -3 | 4 |

*relative to untreated control
**Untreated control has filmcoat green applied.

**Seed treatment disease control efficacy trial**

The crop protection treatments selected for this work were chosen based on their anticipated efficacy, with the rates used provided by the product manufacturers. Many of the treatments reduced the rate of seed germination, although no other significant phytotoxic effects of a commercial concern e.g. seedling stunting, yellowing etc., were observed in the developing seedlings. However, the negative impact of these treatments on seed germination in the absence of Pythium makes the interpretation of the results from the artificially inoculated trial challenging given that one main effect of the pathogen is to cause pre-emergence damping off.

**Emergence**

- Uninoculated control seed emergence was 53.1% at the final assessment (23 days after sowing)
- Emergence increased in all treatments during course of trial.
- Untreated inoculated had 38.5% emergence – disease pressure was sufficient to cause damping off.
- No seed treatment significantly increased rate of emergence compared with untreated, inoculated control ($p>0.05$).
- AHDB9797 resulted in significant reduction ($p<0.05$) in seedling emergence at assessments 2 and 5 compared to the inoculated control. Likely due to treatment, rather than disease. Germination data in absence of Pythium showed reduced emergence.
- AHDB9848 significantly reduced compared with uninoculated control at assessment 2, but by final assessment it was not significantly different ($p>0.05$) from the untreated, inoculated control.

**Table 3**. Effect of plant protection product <u>seed treatments</u> on the mean seedling emergence (%) for leek seed grown in soil artificially inoculated with *P. ultimum* at five assessment dates.

| Date / Treatment | 02 April | 05 April | 07 April | 12 April | 16 April | % difference vs untreated inoculated control (16 Apr) |
|---|---|---|---|---|---|---|
| Uninoculated control | 9.4 | 29.2 | 38.5 | 40.6 | 53.1 | |
| Inoculated control | 5.2 | 20.8 | 25 | 27.1 | 38.5 | |
| AHDB9797 | 0 | **2.1** | 5.2 | 5.2 | 11.5 | -70.1 |
| AHDB9849 | 4.2 | 18.7 | 21.9 | 24 | 35.4 | -8.1 |
| AHDB9815 | 5.2 | 19.8 | 29.2 | 30.2 | 41.7 | 8.3 |
| AHDB9941 | 11.5 | 26 | 29.2 | 32.3 | 41.7 | 8.3 |
| AHDB9848 | 1 | **9.4** | 17.7 | 21.9 | 45.8 | 19.0 |
| AHDB9955 | 2.1 | 14.6 | 19.8 | 26 | 34.4 | -10.6 |
| P value | 0.076 | 0.037 | 0.075 | 0.094 | 0.034 | |
| d.f. | 21 | 21 | 21 | 21 | 21 | |
| s.e.d. | 3.79 | 7.56 | 9.35 | 10.02 | 10.4 | |
| l.s.d. | 7.87 | 15.72 | 19.45 | 20.84 | 21.64 | |
| (orange) | Significantly different from untreated inoculated control (p>0.05) | | | | | |
| (blue) | Not significantly different from untreated inoculated control (p>0.05) | | | | | |

Values in bold represent significant reduction in mean seedling emergence compared with the uninoculated control.

**Pythium incidence**

- Pythium formed mats on soil surface by final assessment date (16/04/21).
- Inoculated control resulted in 7.3% diseased seedlings.
- The greatest increase in disease incidence was between assessments 4 and 5.
- AHDB9797 – no symptoms of damping off noted until last assessment, when it was the lowest of all treatments.
- No treatments significantly reduced the incidence of Pythium.
- AHDB9848 and AHDB9941 resulted in increased Pythium incidence (both 12.5%) compared with inoculated control by final assessment, although not significant.
- Despite AHDB9797 being phytotoxic to treated seed, it may have provided some protection for seeds that did grow against Pythium at the rate used. The phytotoxicity renders it of no commercial use.
- By the final assessment, the incidence of Pythium increased in some treatments compared with the inoculated control e.g. AHDB9848 and AHDB9941 (both 12.5%), but not significantly.

**Table 4.** Effect of plant protection product seed treatments on Pythium incidence (%) at five assessment dates for leek seed grown in soil artificially inoculated with *P. ultimum*.

| Date / Treatment | 02-Apr | 05-Apr | 07-Apr | 12-Apr | 16-Apr | % difference vs untreated inoculated control 16 Apr |
|---|---|---|---|---|---|---|
| Uninoculated control | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | |
| Inoculated control | 0.0 | 3.1 | 5.2 | 5.2 | 7.3 | |
| AHDB9797 | 0.0 | 0.0 | 0.0 | 0.0 | 4.2 | -42.5 |
| AHDB9849 | 0.0 | 2.1 | 5.2 | 6.3 | 7.3 | 0.0 |
| AHDB9815 | 0.0 | 2.1 | 6.3 | 6.3 | 10.4 | 42.5 |
| AHDB9941 | 0.0 | 1.0 | 7.3 | 7.3 | 12.5 | 71.2 |
| AHDB9848 | 0.0 | 1.0 | 2.1 | 3.1 | 12.5 | 71.2 |
| AHDB9955 | 0.0 | 0.0 | 3.1 | 3.1 | 4.2 | -42.5 |
| P value | - | 0.274 | 0.01 | 0.011 | 0.164 | |
| d.f. | - | 21 | 21 | 21 | 21 | |
| s.e.d. | - | 1.42 | 2.06 | 2.12 | 4.8 | |
| l.s.d. | - | 2.96 | 4.28 | 4.41 | 9.98 | |
| (orange) | Significantly different from untreated inoculated control (p>0.05) | | | | | |
| (blue) | Not significantly different from untreated inoculated control (p>0.05) | | | | | |

**Pythium severity**

- AHDB9797 had significantly less disease than the inoculated control at assessment 3.
- AHDB9849 had significantly higher disease than other treatments at assessments 3 and 4.

**Table 5.** Effect of different plant protection product seed treatments at five assessment dates on Pythium severity (%) in leek seed grown in soil artificially inoculated with *P. ultimum*.

| Date / Treatment | 02-Apr | 05-Apr | 07-Apr | 12-Apr | 16-Apr | % difference vs untreated inoculated control 16 Apr |
|---|---|---|---|---|---|---|
| Uninoculated control | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | |
| Inoculated control | 0.0 | 7.5 | 11.3 | 11.3 | 66.0 | |
| AHDB9797 | 0.0 | 0.0 | 0.0 | 0.0 | 44.0 | -33.3 |
| AHDB9849 | 0.0 | 15.0 | 27.5 | 35.0 | 66.0 | 0.0 |
| AHDB9815 | 0.0 | 5.0 | 8.7 | 8.8 | 66.0 | 0.0 |
| AHDB9941 | 0.0 | 2.5 | 10.0 | 10.0 | 22.0 | -66.7 |
| AHDB9848 | 0.0 | 2.5 | 7.5 | 12.5 | 44.0 | -33.3 |
| AHDB9955 | 0.0 | 0.0 | 5.0 | 7.5 | 25.1 | -62.0 |
| P value | - | 0.407 | <0.001 | 0.005 | 0.194 | |
| d.f. | - | 21 | 21 | 21 | 21 | |
| s.e.d. | - | 7.01 | 3.76 | 7.57 | 27.46 | |
| l.s.d. | - | 14.58 | 7.819 | 15.74 | 57.11 | |
| (orange) | Significantly different from untreated inoculated control (p>0.05) | | | | | |

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

**Seedling vigour**

- The uninoculated control was used as a 100% vigour benchmark.
- Vigour for all treatments was generally low (<75%) until the final assessment, with exception of AHDB9941.
- AHDB9797 had significantly reduced vigour at assessments 1 and 2 compared to the inoculated control and emergence was also low. It also had the lowest final vigour, although not significantly different from other treatments.

**Table 6.** Effect of plant protection product seed treatments at five assessment dates on the vigour of leek seedlings grown in soil artificially inoculated with *P. ultimum*.

| Date<br>Treatment | 02-Apr | 05-Apr | 07-Apr | 12-Apr | 16-Apr | % difference vs untreated inoculated control 16 Apr |
|---|---|---|---|---|---|---|
| Uninoculated control | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | |
| Inoculated control | 68.8 | 65.0 | 68.8 | 68.8 | 92.5 | |
| AHDB9797 | **0.0** | **12.5** | **32.5** | 45.0 | 62.5 | -32.4 |
| AHDB9849 | 60.0 | **58.8** | **52.5** | 52.5 | 77.5 | -16.2 |
| AHDB9815 | 70.0 | **52.5** | 63.8 | 75.0 | 95.0 | 2.7 |
| AHDB9941 | 57.5 | 95.0 | 93.8 | 92.5 | 100.0 | 8.1 |
| AHDB9848 | **18.8** | **30.0** | **25.0** | 35.0 | 85.0 | -8.1 |
| AHDB9955 | **37.5** | **55.0** | **25.8** | 52.5 | 80.0 | -13.5 |
| P value | 0.02 | 0.003 | 0.009 | 0.05 | 0.193 | |
| d.f. | 21 | 21 | 21 | 21 | 21 | |
| s.e.d. | 25.37 | 19.51 | 19.23 | 20.65 | 14.52 | |
| l.s.d. | 52.76 | 40.58 | 39.99 | 42.95 | 30.2 | |
| | Significantly different from untreated inoculated control (p>0.05) | | | | | |
| | Not significantly different from untreated inoculated control (p>0.05) | | | | | |

Values in bold represent significant reductions in seedling vigour compared with the uninoculated control.

**Fresh weight**

- No significant differences in fresh weight were recorded between treatments, the inoculated, control and uninoculated control seedlings (p=0.069).

## Conclusions

- No seed treatment significantly reduced damping off in leek.
- The conventional products AHDB9941 and AHDB9848 had the lowest impact on germination (at both early and late germination tests) suggesting these treatments have no long-term impact on seed viability. The efficacy of these products against Pythium could be revisited using greater application rates.
- AHDB9797 treatment appeared to reduce the incidence and severity of Pythium but seedling emergence was low due to phytotoxic effects on seed germination.

- Bioprotectants, including AHDB9849, AHDB9955 and AHDB9815 may work better under lower inoculum loads and their efficacy could be further investigated under natural infestation conditions when Pythium is likely to build up more gradually.

## Summary

Damping-off is a condition caused by different species of pathogenic fungi and oomycetes which leads to die back of infected seedlings. In leek, *Pythium ultimum*, an oomycete, is one of the pathogens responsible for damping-off. The disease affects the roots and is characterized by seed rotting, failed emergence or the death of seedlings soon after they emerge. More established plants, or those grown under a lower inoculum load, may be able to grow through and survive infection with Pythium, however their growth rate is often reduced.

Seed treatment is an important part of managing Pythium in leek. Until recently Thiram was the standard seed treatment product used, applied to the seed as a warm water soak. With the withdrawal of Thiram, alternative seed treatments are required to minimize the threat from *P*. *ultimum*. 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 was conducted under controlled environmental conditions with treated seeds sown in soil-based growing-media artificially infested with *P. ultimum* to test disease control efficacy. Preceding this, germination trials were conducted with treated seed shortly after, and following 12 weeks of storage, to determine any potential phytotoxicity of the treatments.

## Objectives

1. To evaluate fungicides and bioprotectants as potential seed treatments for efficacy against damping off caused by Pythium (*P. ultimum)* infection of leek.
2. To assess crop safety of seed treatments 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(4) | Design and analysis of efficacy evaluation trials | None |
| PP 1/135(4) | Phytotoxicity assessment | None |
| PP 1/181(4) | Conduct and reporting of efficacy evaluation trials including 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 |
| PP 1/224(2) | Principles of efficacy evaluation for minor use | None |
| PP 1/225(2) | Minimum effective dose | None |

## Test site

| **Item** | **Details** |
|---|---|
| Location address | ADAS Boxworth Pathology Laboratory, Cambridge CB23 4NN |
| Crop | Leek |
| Cultivar | Porvite |
| Agronomic practice | N/A |
| Prior history of site | N/A |

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

## Trial design

1) Seed phytotoxicity – immediately after and 12 weeks after seed treatments were applied.
2) Determination of optimal Pythium concentration for inoculation studies.
3) Disease control efficacy – treated seeds sown into *P. ultimum* inoculated growing-media.

### 1. Seed phytotoxicity germination trials

Germination trials were established to test the potential impact of seed treatment on germination. Germination was assessed shortly after seed treatment was applied by Elsoms Seeds Ltd, and then at ADAS Boxworth after the seed had been stored for 12 weeks under chilled (ca. 5$^{o}$C), dark conditions replicating medium term commercial storage conditions.

| Item | Details |
|---|---|
| Trial design: | Randomised |
| Number of replicates: | 1 |
| Plot size: | Plastic trays |
| Plot size: (cm$^2$): | 201.25 (17.5 x 11.5) |
| Number of seeds per plot: | 100 |
| Number of seeds per treatment: | 400 |
| Leaf Wall Area Calculations: | N/A |

### 2. Determination of inoculum concentration tests

Preliminary work established the optimal inoculum concentration to achieve sufficient damping off for differences between seed treatments and the untreated controls to potentially be observed.

| Item | Details |
|---|---|
| Trial design: | Randomised |
| Number of inoculum rates | 4 |
| 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 |

### 3. Artificially inoculated efficacy trial

Treated seed was grown in soil-based growing-media artificially inoculated with *P. ultimum* to establish the efficacy of the treatments in reducing symptoms of damping off disease.

| Item | Details |
|---|---|
| Trial design | Randomised |
| Number of replicates | 4 |
| Plot size: | Plastic trays |
| Plot size: (cm$^2$): | 201.25 (17.5 x 11.5) |
| Number of plants per plot: | 24 (3 x 8 pattern) |
| Number of plants 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 |
|---|---|---|---|---|---|
| N/A | N/A | Untreated | N/A | N/A | N/A |
| AHDB9797 | N/D | N/D | 18015/001 | N/D | Wettable powder |
| AHDB9849 | N/D | N/D | 0022760086 | N/D | Flowable concentrate |
| AHDB9815 | N/D | N/D | 18062020 | N/D | Wettable powder |
| AHDB9941 | N/D | N/D | EM4L022450 | N/D | Soluble concentrate |
| AHDB9848 | N/D | N/D | 2018-005011 | N/D | Flowable concentrate |
| AHDB9955 | N/D | N/D | 08191930 | N/D | Wettable powder |

# Methods, assessment and records

Efficacy and crop safety experiments were conducted with leek seed of the susceptible variety, Porvite. Seeds were treated with 6 different crop protection products by Elsom's Ltd. All seeds also received Filmcoat Green which is a standard commercial practice.

## Seed preparation

*Seed treatment*

Approximately 1.5 kg of untreated leek seed of the susceptible variety, Porvite (1000 seed weight – 3.2 g), was obtained from a commercial producer.  All seeds were sterilized in the pathology laboratory at ADAS Boxworth before being sent to Elsom's Seeds Ltd. For treatment. In addition to the treatments all seeds were coated with a polymer film (Filmcoat Green) which is standard commercial practice.

*Surface sterilization*

All seeds were surface sterilized before treatments were applied. Seeds were soaked in a 1% sodium hypochlorite solution for 30 seconds, followed by three 1-minute rinses in sterile distilled water. Sterilised seeds were dried in a laminar flow hood and packaged. Seed was stored in sealed plastic bags the dark under cool (ca. 5ºC), 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 were sampled for each treatment, including controls. Sampled seeds were stored in labelled paper bags, stored under dark, cold (ca. 5ºC), dry conditions.

*Seed treatment application – Elsoms Seeds Ltd.*

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

*Application details*

| Treatment number | Treatment: product name or AHDB code | Rate of active substance (ml or g a.s./ha) | Rate of product (l or kg of seed) | Application code |
|---|---|---|---|---|
| 1 | Untreated* | N/A | N/A | A |
| 2 | AHDB9797 | N/A, seed treatment | 120 g/l | A |
| 3 | AHDB9849 | N/A, seed treatment | 1.6 g/kg | A |
| 4 | AHDB9815 | N/A, seed treatment | 5 g/kg | A |
| 5 | AHDB9941 | N/A, seed treatment | 1.5 ml / kg | A |
| 6 | AHDB9848 | N/A, seed treatment | 10 ml / kg | A |
| 7 | AHDB9955 | N/A, seed treatment | 40 g/kg | A |

*all seed, including untreated seed were coated with the polymer Filmcoat Green to replicate standard commercial practice.

### 1. Seed phytotoxicity germination tests

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 week post treatment by Elsoms Seeds Ltd and 12 weeks post-treatment by ADAS Horticulture.

**a. 1 week post treatment – conducted by Elsoms Seeds Ltd**

- 100 seeds/treatment.
- Seeds were sown onto moist filter paper in sealed plastic boxes
- Scored for germination at 28 days
- Seeds scored as germinated or ungerminated (counts).

**b. 12 weeks post treatment – conducted by ADAS Horticulture**

- 12 weeks represents medium-term commercial storage practices.
- 100 seeds subsampled from each seed batch.
- Seeds sown onto moist filter papers in plastic trays with lids in 20x5 grid.
- Incubated in controlled environment cabinet at 20ºC with a 16:8 hour light: dark cycle for 21 days.
- Boxes were checked every 2-3 days to ensure the filter paper remained moist.
- Scored for emergence at 7 and 15 days.
- Scored for seedling quality (1-5 scale) at 15 days.

At 12 weeks those that germinated were also visually assessed for average seedling quality using the rating scale on a whole plot basis:

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.

Note: The early toxicity germination test conducted by Elsom's Seeds Ltd. did not include the assessment of seedling quality.

## 2. Determination of optimal *P. ultimum* inoculum rate

A preliminary study was conducted to determine optimal *P. ultimum* inoculum concentration to use for inoculation experiments providing sufficient disease pressure to induce symptoms but not so high as to overwhelm all treatments i.e. approximately 50% plant death.

### *Culture preparation and Growing media inoculation*

*P. ultimum* cultures were grown on cornmeal agar for seven days (20ºC, 16:18 light: dark cycle). Agar plugs from growing cultures were transferred to conical flasks containing 100 ml of sterile potato dextrose broth and stored for a further seven days in dark conditions at 25ºC until thick mycelial mats had formed on the broth surface. John Innes No. 1 growing-media, amended with 1% w/w ground oatmeal to aid growth of the culture throughout. This mix was autoclaved twice for 2 hours at 121ºC to sterilize.

Pythium mats were mixed to form a thick slurry which was then combined with the sterilized growing-media to produce the inoculated growing media.
Different numbers of mycelial mats (0.0, 0.5, 1.0 and 2.0 mats per 1.2 kg growing media) were incorporated into the artificially inoculated growing- media was tested.

This was determined to be a 1 mat of Pythium per 1.2 kg John Innes No. 1 seedling compost.

### *Seed sowing*

60 seeds were sown into inoculated growth media prepared as described above with different Pythium concentrations (0.0, 0.5, 1.0 and 2.0 mycelium mats) and incubated at 20ºC with a 16:8 hour light: dark cycle for 21 days.

*Seed germination assessment*
Pre-emergence damping off was assessed by counting the number of germinated seeds at 7 and 14 days after germination started.

The optimal rate of inoculum was defined at that which gave ~ 50% plants losses (failure of seedling emergence) in the inoculated control compared with the untreated control.

## 3. Evaluation of seed treatment efficacy

To test the disease control efficacy of the seed treatments, they were challenged in a controlled experiment by sowing into growing medium inoculated with a pathogenic isolate of *P. ultimum*. Seed sowing and growing conditions were as described above.

Seed trays were filled with 200 g of growing media (uninoculated control or inoculated) to a depth of 5 cm. Four replicates of 24 seeds were sown into growth medium for each treatment in an 8 x 3 grid pattern. Lids were placed on the seed trays to maintain humidity and the trays were placed in controlled environment cabinet following a randomized block design. Seeds were incubated for 28 days (20ºC, 16:18 light dark cycle, 80% RH) and assessed at 5 time points up to 25 days. Once emergence of seedlings had started the lids were removed.

At each assessment point the following assessments were made:
1. Number of emerged seedlings.
2. Number of seedlings failing to emerge.
3. Disease incidence % - visual assessment
4. Disease severity % - visual assessment
5. Seedling vigour % - assessed relative to uninoculated control (setting the control as a 100% vigorous benchmark). Missing plants were not included.

At the final assessment, a destructive assessment was completed, and the fresh weight (g) of each plot calculated after the plants were gently washed, blotted and weighed.

*Assessment schedule*

| Assessment no. | Assessment date | Seedling emergence in the uninoculated control (%) | Seedling emergence in the inoculated control (%) |
|---|---|---|---|
| 1 | 02 April | 9.4 | 5.2 |
| 2 | 05 April | 29.2 | 20.8 |
| 3 | 07 April | 38.5 | 25.0 |
| 4 | 12 April | 40.6 | 27.1 |
| 5 | 16 April | 53.1 | 38.5 |

*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 Pythium | *Pythium ultimum* | PYTHUL | Absent | 0% incidence | 7.3% incidence |

*Statistical analysis*
The germination tests and efficacy trial were laid out as a randomised complete block design. Statistical analysis was carried out using ANOVA in Genstat 18. To assess for differences between treatments compared with the untreated control, disease incidence and severity values were used as variables to determine efficacy, and the different classifications of germinated seed were used as variables to determine phytotoxicity. No data transformation was required. Statistical analysis was performed by the ADAS statistician Chris Dyer.

## Results

### Seed phytotoxicity germination tests

**a. 1 week post treatment – conducted by Elsoms Seeds Ltd**

This trial was conducted by Elsom's Seeds Ltd shortly after seed treatment (26 February 2021). Germination was scored as having occurred or not. Seedling quality and vigour were not recorded.

Table 7**.** Germination of seeds immediately after treatment, February-March 2021

| Treatment code | Treatment | Germination % final | % difference in germination* |
|---|---|---|---|
| 1 | Untreated | 64 | |
| 2 | Filmcoat green | 62 | -3 |
| 3 | AHDB9797 | 53 | -17 |
| 4 | AHDB9849 | 53 | -17 |
| 5 | AHDB9815 | 48 | -25 |
| 6 | AHDB9941 | 60 | -6 |
| 7 | AHDB9848 | 58 | -9 |
| 8 | AHDB9955 | 49 | -23 |

*Relative to untreated control

This early assessment (Table 7) demonstrated the effects of treatment on seed germination. Replicate results were not provided, and so statistical comparison was not done. Overall, germination was lower than expected in the untreated seed (64%) and the addition of the seed coat Filmcoat Green did not impact this (62%). All treatments (at the rates applied) reduced germination to some extent, with AHDB9941, and AHDB9848 having the least impact (60% and 58% germination respectively). AHDB9815, a bioprotectant product had the greatest impact on germination (48%), whilst AHDB9797 and AHDB9849 both decreased germination to 53%. The untreated control itself has poor germination, which would be commercially unacceptable. It is hard to assess whether the treatments would still be commercially viable with this poor germination as a baseline, but in these tests the treatments have reduced germination further.

**a. 12 weeks post treatment – conducted by ADAS Horticulture**

This trial was conducted at ADAS Boxworth. Seeds were scored as germinated/ungerminated at two timepoints after the first untreated seeds started germinating (7 and 15 days) and scored for seedling quality on a 1-5 scale. Untreated seed which were not coated in Filmcoat Green were not included.

Table 8. Medium term storage of seeds (12 weeks). Germination and seedling quality at 7 and 15 days after sowing.

| | 7 d | | | 15 d | | |
|---|---|---|---|---|---|---|
| **Treatment** | **Germination %** | **% difference in germination*** | **Seedling quality (1-5 index)** | **Germination %** | **% difference in germination*** | **Seedling quality (1-5 index)** |
| Untreated control** | 53 | | 4.5 | 56 | | 4.5 |
| AHDB9797 | 10 | -81 | 4 | 14 | -75 | 4 |
| AHDB9849 | 48 | -9 | 4.5 | 52 | -29 | 5 |
| AHDB9815 | 8 | -85 | 4 | 43 | -25 | 4 |
| AHDB9941 | 57 | 8 | 3.5 | 60 | 9 | 5 |
| AHDB9848 | 57 | 8 | 4 | 61 | 8 | 4.5 |
| AHDB9955 | 52 | 2 | 4.5 | 54 | -3 | 4 |

*relative to untreated control
**Untreated control has filmcoat green applied.

The untreated control (with only filmcoat green applied) had a germination rate of 53% after 7d. There was relatively little increase in germinated seed at the next assessment after 15d. Treatments AHDB9849, AHDB9941 and AHDB9848 behaved similarly with more than a 3% increase in germination by day 15.. However, AHDB9815 and AHDB9797 appeared to have a delay in germination with just 8% and 10% of seeds germinating in the first week representing a 85% and 81% reduction in germination compared with the untreated control. In the case of AHDB9797 germination continued to be low indicating a phytotoxic effect of the treatment. However AHDB 9815 had a marked increase in germinated seeds by the 14d assessment, increasing up to 43%.

**Results were similar to those observed in the early germination tests, apart from AHDB9797 which reduced germination more from 53% in the early germination test (Table 7) to just 14% by day 15 in this trial after 12 weeks seed storage (Table 8**
Table 2). The germination of untreated seed was lower than that recorded at the earlier germination test (62% vs 56%). However, more seed might have germinated if the duration of the trials was increased.

At 15 days, germination was not reduced in the other treatments compared with the earlier assessment and increased slightly in seed treated with AHDB9848 (61% vs. 58%) and AHDB9955 (54% vs. 49%) (Table 1 7 & Table 8). No reductions in germination with longer seed storage were observed in seed treated with AHDB9941 (60%) and AHDB9849 (52%) suggesting these products, at the rates tested, have no long-term impact on germination. A moderate reduction in germination was recorded in seed treated with the biological product AHDB9815; down from 48% to 43%.

At the 7-day assessment, AHDB9941 had a lower seedling quality index than the other treatments, these being the same or only slightly poorer than those with only Filmcoat Green (Table 8).

At the 15-day assessment, only seeds treated with the AHDB9955 had shown a slight quality reduction, while three others had improved slightly (Table 8). The untreated seed received a quality score of 4.5. Although these plants appeared vigorous, there was variation in root size at both 7 and 15 days, with many below 1.0 cm in length. The quality of the roots and seedlings developing from seed treated with AHDB9848 were indistinguishable from the untreated and scored 4.5. The quality of seedlings treated with AHDB9941 and the biological product AHDB9849 received the maximum quality of 5.0, with all roots greater than 1.0 cm in length. In all other metrics the vigour of these seedlings was identical to the untreated.

AHDB9797 and AHDB9815 treated seed stored for 12 weeks before sowing had the lowest quality scores (4.0) because of the reduced germination rate and subsequent delay in root development. The developing cotyledons appeared healthy but were smaller when compared with the other treatments. Given longer it is anticipated that the quality of these plants would be comparable to the other treatments. and so not have a significant long-term impact on quality.

## Evaluation of seed treatment efficacy

The treatments selected for this work were chosen based on their anticipated efficacy, with the rates used provided by the product manufacturers. Many of the treatments reduced the rate of seed germination, however no other significant phytotoxic effects of a commercial concern e.g. seedling stunting, yellowing etc., were observed in the developing seedlings. The negative impact of these treatments on seed germination in the absence of Pythium makes the interpretation of the results from the artificially inoculated trial challenging.

1.Seedling emergence

Seedling emergence increased in all treatments over the course of the trial, with an emergence rate of 53.1% recorded in the uninoculated control seedlings at the final assessment 23 days after sowing (Table 9). As expected, the untreated seed sown in soil-based growing-media inoculated with *P. ultimum* (inoculated control) had a lower emergence rate (38.5%) demonstrating that the disease pressure was sufficient to cause damping off.

No seed treatment significantly increased the rate of seedling emergence compared with the inoculated, untreated control at any assessment date ($p>0.05$). However, at assessments 2 and 5, significant reductions ($p<0.05$) in seedling emergence were found between seed treated with AHDB9797 and the inoculated control (2.1% and 11.5% vs. 20.8% and 38.5% respectively), however is a more likely to be a treatment effect, than a disease related effect (see earlier germination results).

At assessment 2, the number of emerged seedlings was significantly reduced in seedlings treated with AHDB9848 when compared with the uninoculated control. By the final assessment seedling emergence was greatest in this seed treatment. However, this was not significantly greater ($p>0.05$) than the emergence of untreated seedlings grown in the inoculated soil.

At assessment 5 AHDB9848 performed best with 19% better emergence than the untreated inoculated control, but this was not statistically significant.

**Table 9**. Effect of plant protection products on the mean seedling emergence (%) of leek seed grown in soil artificially inoculated with *P. ultimum,* at five assessment dates.

| Date / Treatment | 02 April | 05 April | 07 April | 12 April | 16 April | % difference vs untreated inoculated control (16 Apr) |
|---|---|---|---|---|---|---|
| Uninoculated control | 9.4 | 29.2 | 38.5 | 40.6 | 53.1 | |
| Inoculated control | 5.2 | 20.8 | 25 | 27.1 | 38.5 | |
| AHDB9797 | 0 | **2.1** | 5.2 | 5.2 | 11.5 | -70.1 |
| AHDB9849 | 4.2 | 18.7 | 21.9 | 24 | 35.4 | -8.1 |
| AHDB9815 | 5.2 | 19.8 | 29.2 | 30.2 | 41.7 | 8.3 |
| AHDB9941 | 11.5 | 26 | 29.2 | 32.3 | 41.7 | 8.3 |
| AHDB9848 | 1 | **9.4** | 17.7 | 21.9 | 45.8 | 19.0 |
| AHDB9955 | 2.1 | 14.6 | 19.8 | 26 | 34.4 | -10.6 |
| P value | 0.076 | 0.037 | 0.075 | 0.094 | 0.034 | |
| d.f. | 21 | 21 | 21 | 21 | 21 | |
| s.e.d. | 3.79 | 7.56 | 9.35 | 10.02 | 10.4 | |
| l.s.d. | 7.87 | 15.72 | 19.45 | 20.84 | 21.64 | |
| | Significantly different from untreated inoculated control (p>0.05) | | | | | |

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

Values in bold represent significant reduction in mean seedling emergence compared with the uninoculated control.

1. Pythium incidence

Pythium incidence increased over time reaching 7.3% in the inoculated control by the final assessment, with Pythium presence confirmed microscopically. During the trial, Pythium continued to grow within the inoculated soil and had formed mats on the soil surface by the final assessment. This correlated with a sudden increase in pythium incidence in most treatments.

Low levels of seedlings emerged from seeds treated with AHDB9797 by the second assessment (05 April, 2.1% Table 9), however no symptoms of Pythium were found in this treatment until the final assessment (16 April, Table 10). Significant reductions in pythium incidence were recorded at assessment 3 (p<0.05), but as the number of emerged seedlings was low at this time (5.2%), the validity of this reduction must be examined critically. Despite being phytotoxic to the treated seed, the rate of AHDB9797 tested could potentially have been sufficient to protect the few seedlings that did emerge and grow. If this were the case it would demonstrate efficacy against Pythium but would be of no commercial value to growers.

No other treatment significantly reduced the incidence of Pythium at any assessment. By the final assessment, the incidence of Pythium increased in some treatments compared with the inoculated control e.g. AHDB9848 and AHDB9941 (both 12.5%), but not significantly from that in the inoculated control.

**Table 10**. Effect of plant protection products on Pythium incidence (%) in leek seed grown in soil artificially inoculated with *P. ultimum,* at five assessment dates.

| Date / Treatment | 02-Apr | 05-Apr | 07-Apr | 12-Apr | 16-Apr | % difference vs untreated inoculated control 16 Apr |
|---|---|---|---|---|---|---|
| Uninoculated control | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | |
| Inoculated control | 0.0 | 3.1 | 5.2 | 5.2 | 7.3 | |
| AHDB9797 | 0.0 | 0.0 | 0.0 | 0.0 | 4.2 | -42.5 |
| AHDB9849 | 0.0 | 2.1 | 5.2 | 6.3 | 7.3 | 0.0 |
| AHDB9815 | 0.0 | 2.1 | 6.3 | 6.3 | 10.4 | 42.5 |
| AHDB9941 | 0.0 | 1.0 | 7.3 | 7.3 | 12.5 | 71.2 |
| AHDB9848 | 0.0 | 1.0 | 2.1 | 3.1 | 12.5 | 71.2 |
| AHDB9955 | 0.0 | 0.0 | 3.1 | 3.1 | 4.2 | -42.5 |
| P value | - | 0.274 | 0.01 | 0.011 | 0.164 | |
| d.f. | - | 21 | 21 | 21 | 21 | |
| s.e.d. | - | 1.42 | 2.06 | 2.12 | 4.8 | |
| l.s.d. | - | 2.96 | 4.28 | 4.41 | 9.98 | |
| | Significantly different from untreated inoculated control (p>0.05) | | | | | |
| | Not significantly different from untreated inoculated control (p>0.05) | | | | | |

2. Pythium severity

The inoculated control disease severity increased through time to reach 66% by the final assessment and this was observed for all treatments. This corresponded with the increased disease incidence and growth of Pythium mats on the surface of the inoculated growing-media (Table 11).

Pythium severity reflected incidence, with significant reductions recorded in the AHDB9797 treated seed at assessment 3 (0.0%) compared with the inoculated control (11.3%). No other instances of reductions in disease severity were recorded, however significant increases in Pythium severity had developed in seed treated with AHDB9849 by assessments 3 and 4 (27.5% and 35.0% respectively).

**Table 11**. Effect of different plant protection products on pythium severity (%) in leek seed grown in soil artificially inoculated with *P. ultimum* at five assessment dates.

| Date / Treatment | 02-Apr | 05-Apr | 07-Apr | 12-Apr | 16-Apr | % difference vs untreated inoculated control 16 Apr |
|---|---|---|---|---|---|---|
| Uninoculated control | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | |
| Inoculated control | 0.0 | 7.5 | 11.3 | 11.3 | 66.0 | |
| AHDB9797 | 0.0 | 0.0 | 0.0 | 0.0 | 44.0 | -33.3 |
| AHDB9849 | 0.0 | 15.0 | 27.5 | 35.0 | 66.0 | 0.0 |
| AHDB9815 | 0.0 | 5.0 | 8.7 | 8.8 | 66.0 | 0.0 |
| AHDB9941 | 0.0 | 2.5 | 10.0 | 10.0 | 22.0 | -66.7 |
| AHDB9848 | 0.0 | 2.5 | 7.5 | 12.5 | 44.0 | -33.3 |
| AHDB9955 | 0.0 | 0.0 | 5.0 | 7.5 | 25.1 | -62.0 |
| P value | - | 0.407 | <0.001 | 0.005 | 0.194 | |
| d.f. | - | 21 | 21 | 21 | 21 | |
| s.e.d. | - | 7.01 | 3.76 | 7.57 | 27.46 | |
| l.s.d. | - | 14.58 | 7.819 | 15.74 | 57.11 | |
| (orange) | Significantly different from untreated inoculated control (p>0.05) | | | | | |
| (blue) | Not significantly different from untreated inoculated control (p>0.05) | | | | | |

3. Seedling vigour

Seedling vigour was recorded with the uninoculated control used as a 100% vigour benchmark. Vigour in all treatments, apart from AHDB9941 was low for most of the trial period (below 75%), until the final assessment (Table 12). At this time, the vigour of all treatments, apart from AHDB9797 had recovered and were not statistically different to either the inoculated, or uninoculated untreated controls (p=0.193). This suggest the surviving seedlings were growing through the infection.

Seedling vigour was significantly lower in seed treated with AHDB9797 than the inoculated control at assessments 1 and 2 (p<0.05) and few seedlings had emerged, indicating that this treatment had the most severe impact on seed of any tested.

**Table 12**. Effect of plant protection products on the vigour (%) of leek seedlings grown in soil artificially inoculated with *P. ultimum,* at five assessment dates.

| Date<br>Treatment | 02-Apr | 05-Apr | 07-Apr | 12-Apr | 16-Apr | % difference vs untreated inoculated control 16 Apr |
|---|---|---|---|---|---|---|
| Uninoculated control | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | |
| Inoculated control | 68.8 | 65.0 | 68.8 | 68.8 | 92.5 | |
| AHDB9797 | **0.0** | **12.5** | **32.5** | 45.0 | 62.5 | -32.4 |
| AHDB9849 | 60.0 | **58.8** | **52.5** | 52.5 | 77.5 | -16.2 |
| AHDB9815 | 70.0 | **52.5** | 63.8 | 75.0 | 95.0 | 2.7 |
| AHDB9941 | 57.5 | 95.0 | 93.8 | 92.5 | 100.0 | 8.1 |
| AHDB9848 | **18.8** | **30.0** | **25.0** | 35.0 | 85.0 | -8.1 |
| AHDB9955 | **37.5** | **55.0** | **25.8** | 52.5 | 80.0 | -13.5 |
| P value | 0.02 | 0.003 | 0.009 | 0.05 | 0.193 | |
| d.f. | 21 | 21 | 21 | 21 | 21 | |
| s.e.d. | 25.37 | 19.51 | 19.23 | 20.65 | 14.52 | |
| l.s.d. | 52.76 | 40.58 | 39.99 | 42.95 | 30.2 | |
| (orange) | Significantly different from untreated inoculated control (p>0.05) | | | | | |
| (blue) | Not significantly different from untreated inoculated control (p>0.05) | | | | | |

Values in bold represent significant reduction in seedling vigour compared with the uninoculated control.

4. Fresh weight

No significant differences in fresh weight were recorded between treatments, the inoculated control and uninoculated control seedlings (p=0.069).

## Discussion

Germination of the untreated leek seed was lower than anticipated in both the early and late (post-storage) germination trials, at 64% and 56% respectively, and these rates would not be considered acceptable to commercial growers. This trial was originally due to take place in 2020, and the age of the seed used may be responsible for the low germination rate. The seed sterilization step could also potentially have reduced germination rates. However the sterilization method has been used successfully on leek seeds in a similar trial with no apparent issues. In the germination trials all treatments (at the rates tested) reduced leek seed germination compared with the untreated control, with many at a level below which is commercially acceptable. For several, this effect was enhanced following 12 weeks of medium-term storage under commercial conditions.

AHDB9941 and AHDB9848 seed treatments had the lowest impact on leek seed germination and did not impact seedling quality at both the early and late germination assessments, suggesting that their effect on germination was minimal. These products could be re-evaluated using higher treatment concentrations which may be effective against Pythium. Other treatments had a greater impact on germination, including the biological product AHDB9815 which also delayed seed germination. AHDB9797 reduced the rate of germination the most after 12 weeks of storage, reducing germination to just 14%.

The optimal inoculum load was determined by the preliminary experiments with different concentrations of inoculum in the growth medium. Seed emergence was recorded but not disease incidence or severity on those emerged seedlings. Thus, pre-emergence damping off was assessed, but not post-emergence damping off. Both measures would have provided determined which symptoms(s) (i.e. pre or post emergence) the isolate of *P. ultimum* used in the experiments caused.

In the efficacy trials using the *P. ultimum* inoculated growing medium, AHDB9797 significantly reduced the number of emerged seedlings compared with the number recorded in the inoculated control at two assessments. However, this is believed to a phytotoxic effect associated with this treatment rather than pathogen-related. AHDB9797 also reduced the incidence and severity of Pythium at assessment 3. However, this is confounded by the low number of emerged seedlings (11.5% at the final assessment), and it is not possible to say with confidence that the reduction in Pythium severity is due to the smaller sample size, or the treatment rate tested. Investigating this effect further is unwarranted due to the negative effect of AHDB9797 on seed germination.

To establish the true impact of these products against Pythium, the soil was artificially inoculated with Pythium following growing media preparation and sterilization. Although appropriate to this investigation, this environment is artificial, lacking the natural flora and fauna associated with soil grown crops. Inoculation also swiftly creates a high infestation level in the growing-media. It is possible that the impact of the three bioprotectant products such as AHDB9815, AHDB9849 and AHDB9955 may be improved in a more natural environment. This would require further 'real world' studies, such as grower led demonstration trials.

Soil inoculation with *P. ultimum* in this trial reduced the emergence of untreated seedlings by 27.5%. Although greater rates of damping-off are reported in severely impacted fields, biological products typically work best under low inoculum loads. A repeat of this work under lower disease pressure might demonstrate efficacy which is masked by the trial design.

Pythium severity was significantly higher in seed treated with AHDB9849 than the inoculated control at assessments 3 (7/4/22) and 4 (12/4/22). The cause for this is unclear, however AHDB9849 is a bioprotectant product based on a bacterial species (*Bacillus*) and the presence of this organism on the seedling may have enhanced its susceptibility to Pythium.

No seed treatment at the rates tested gave significant and consistent control of Pythium in these trials. However, both AHDB9941 and AHDB9848 were shown to be the crop safe, and the rates of these products could be adapted in further work to confirm if they provide pythium control.

## Conclusions

Germination:

- The percentage germination of the untreated seed was low and below that which would be commercially acceptable.
- The percentage germination in all seed treatments was lower than that of the untreated control at the early assessment.
- The low germination baseline of the untreated control makes it difficult to assess whether the reduction in germination induced by treatments would make germination rates commercially unviable if the overall performance of the seed was better.
- The percentage germination after 12 weeks of storage of seed treated with the products AHDB9941, AHDB9848, AHDB9849 and AHDB9955 was all above 50% and so equivalent to untreated seed, with seedling quality comparable to, or greater, than the untreated seed.
- AHDB9815 initially delayed germination of a high proportion of the seeds after 12 weeks of storage.
- AHDB9797 significantly impacted germination with only 14% germinated a fortnight after sowing following 12 weeks' storage.

Efficacy

- Preliminary work identified the inoculum rate of Pythium required to cause damping-off in leek.
- Efficacy against Pythium was established by assessing seedling emergence, Pythium incidence and severity, seedling vigour and fresh weight from treated seed grown in soil artificially inoculated with *P. ultimum.*
- Seedling emergence from untreated seed was reduced from 53% to 38%, in the inoculated soil compared with the uninoculated soil, a 27.5% reduction.
- No product significantly increased seedling emergence compared with the inoculated control.
- AHDB9797 treatment appeared to reduce the incidence and severity of Pythium at one assessment, however this was based on the low number of emerged seedlings in this treatment. No other product significantly reduced the incidence or severity of Pythium at any assessment date compared with the inoculated control.
- Initially seedling vigour was reduced in seed grown in soil inoculated with Pythium, however this recovered over time.
- By the final assessment no significant differences were recorded between treatments and the inoculated control.
- No differences in fresh weight were found between treatments and the inoculated control.

Overall

- No seed treatment significantly reduced damping off in leek.
- The conventional products AHDB9941 and AHDB9848 had the lowest impact on germination (at both early and late germination tests) suggesting these treatments have no long-term impact on seed viability. The efficacy of these products against Pythium could be revisited using greater application rates.
- Bioprotectants, including AHDB9849, AHDB9955 and AHDB9815 could work better under lower inoculum loads and their efficacy could be further investigated under natural infestation conditions when Pythium is likely to build up more gradually.

## Acknowledgements

We would like to thank AHDB and the participating crop protection companies for project funding. Thanks to Elsoms for providing seed treatment facilities.

## Appendix

a. Crop diary – events related to growing crop

| Crop | Cultivar | Treatment date |
|---|---|---|
| Leek | Porvite | 26 February 2021 |

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

| Date | Event |
|---|---|
| **Pre-trial** | |
| 16/04/2020 | Pythium cultures subbed from ADAS culture collection |
| 05/05/2020 | Pythium confirmed via microscopy. |
| 18/08/2020 - 19/08/2020 | Seed surface sterilised, air dried, subsampled and bagged. Seed stored in the dark ca. 5ºC |
| 14/09/2020 | *P. ultimum* optimal load tests established |
| 29/09/2020 | Optimal load assessments |
| 26/02/2021 | Seed treatment by Elsoms |
| **Germination tests** | |
| 26/02/2021 | Start of early germination trial at Elsoms |
| 12/05/2021 | Late germination trial set-up at ADAS Boxworth |
| 18/05/2021 | Germination started |
| 25/05/2021 | Germination assessment 1 |
| 01/06/2021 | Germination assessment 2 |
| **Efficacy tests** | |
| 11/03/2021-23/03/2021 | Original trial: Terminated |
| 24/03/2021 | Trial sowed in soil artificially inoculated with *P. ultimum*. |
| 02/04/2021 | Assessment 1: Seedling emergence, disease incidence and severity and vigour |
| 05/04/2021 | Assessment 2: Seedling emergence, disease incidence and severity and vigour |
| 07/04/2021 | Assessment 3: Seedling emergence, disease incidence and severity and vigour |
| 12/04/2021 | Assessment 4: Seedling emergence, disease incidence and severity and vigour |
| 16/04/2021 | Assessment 5 (destructive assessment): Seedling emergence, disease incidence and severity, vigour and fresh weights |

c. Trial plan

**Top shelf**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Plot** | **201** | **202** | **203** | **204** | **205** | **206** | **207** | **208** |
| **Treatment** | **4** | **8** | **3** | **1** | **2** | **6** | **7** | **5** |
| **Block** | **2** | **2** | **2** | **2** | **2** | **2** | **2** | **2** |
| | **Back** | | | | | | | |
| **Plot** | **101** | **102** | **103** | **104** | **105** | **106** | **107** | **108** |
| **Treatment** | **4** | **1** | **8** | **6** | **3** | **5** | **2** | **7** |
| **Block** | **1** | **1** | **1** | **1** | **1** | **1** | **1** | **1** |
| | **Front** | | | | | | | |

**Bottom shelf**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Plot** | **301** | **302** | **303** | **304** | **305** | **306** | **307** | **308** |
| **Treatment** | **7** | **5** | **4** | **2** | **6** | **8** | **3** | **1** |
| **Block** | **3** | **3** | **3** | **3** | **3** | **3** | **3** | **3** |
| | **Back** | | | | | | | |
| **Plot** | **2** | **6** | **1** | **3** | **5** | **4** | **7** | **8** |
| **Treatment** | **4** | **4** | **4** | **4** | **4** | **4** | **4** | **4** |
| **Block** | | | | | | | | |
| | **Front** | | | | | | | |

Treatment table

| **Treatment number** | **AHDB code** |
|---|---|
| 1 | Uninoculated untreated control |
| 2 | Inoculated untreated control |
| 3 | Untreated (Filmcoat Green) |
| 4 | AHDB9797 |
| 5 | AHDB9849 |
| 6 | AHDB9815 |
| 7 | AHDB9941 |
| 8 | AHDB9848 |

d. Raw data from assessments

- Phytotoxicity - germination tests following seed treatment

| Treatment code | Treatment | Germination % final |
|---|---|---|
| 1 | Untreated | 64 |
| 2 | Untreated (Filmcoat Green) | 62 |
| 3 | AHDB9797 | 53 |
| 4 | AHDB9849 | 53 |
| 5 | AHDB9815 | 48 |
| 6 | AHDB9941 | 60 |
| 7 | AHDB9848 | 58 |
| 8 | AHDB9955 | 49 |

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

| Treatment | 25 May | | 01 June | |
|---|---|---|---|---|
| | Germination 7 days | Seedling quality (1-5) | Germination 15 d | Seedling quality (1-5) |
| Untreated (Filmcoat Green) | 53 | 4.5 | 56 | 4.5 |
| AHDB9797 | 10 | 40 | 14 | 4.0 |
| AHDB9849 | 48 | 4.5 | 52 | 5.0 |
| AHDB9815 | 8 | 4.0 | 43 | 4.0 |
| AHDB9941 | 57 | 3.5 | 60 | 5.0 |
| AHDB9848 | 57 | 4.0 | 61 | 4.5 |
| AHDB9955 | 52 | 4.5 | 54 | 4.0 |

- Efficacy trial – Seedling emergence

| Plot | Block | Treatment | 02.04.2021 | 05.04.2021 | 07.04.2021 | 12.04.2021 | 16.04.2021 |
|---|---|---|---|---|---|---|---|
| | | | 02-Apr | 05-Apr | 07-Apr | 12-Apr | 16-Apr |
| 1 | 1 | 4 | 8.3 | 45.8 | 45.8 | 45.8 | 45.8 |
| 2 | 1 | 1 | 4.2 | 20.8 | 20.8 | 20.8 | 37.5 |
| 3 | 1 | 8 | 0.0 | 20.8 | 25.0 | 33.3 | 37.5 |
| 4 | 1 | 6 | 0.0 | 16.7 | 20.8 | 25.0 | 29.2 |
| 5 | 1 | 3 | 0.0 | 8.3 | 8.3 | 8.3 | 8.3 |
| 6 | 1 | 5 | 4.2 | 20.8 | 25.0 | 29.2 | 29.2 |
| 7 | 1 | 2 | 4.2 | 20.8 | 29.2 | 29.2 | 33.3 |
| 8 | 1 | 7 | 0.0 | 4.2 | 4.2 | 4.2 | 12.5 |
| 9 | 2 | 4 | 4.2 | 16.7 | 25.0 | 29.2 | 50.0 |
| 10 | 2 | 8 | 4.2 | 16.7 | 25.0 | 33.3 | 41.7 |
| 11 | 2 | 3 | 0.0 | 0.0 | 4.2 | 4.2 | 4.2 |
| 12 | 2 | 1 | 8.3 | 29.2 | 45.8 | 45.8 | 58.3 |
| 13 | 2 | 2 | 4.2 | 20.8 | 25.0 | 25.0 | 50.0 |
| 14 | 2 | 6 | 12.5 | 33.3 | 33.3 | 37.5 | 54.2 |
| 15 | 2 | 7 | 4.2 | 20.8 | 33.3 | 33.3 | 54.2 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 16 | 2 | 5 | 4.2 | 20.8 | 37.5 | 37.5 | 58.3 |
| 17 | 3 | 7 | 0.0 | 8.3 | 25.0 | 33.3 | 62.5 |
| 18 | 3 | 5 | 0.0 | 12.5 | 12.5 | 12.5 | 20.8 |
| 19 | 3 | 4 | 0.0 | 0.0 | 4.2 | 4.2 | 8.3 |
| 20 | 3 | 2 | 0.0 | 0.0 | 0.0 | 0.0 | 4.2 |
| 21 | 3 | 6 | 4.2 | 8.3 | 8.3 | 8.3 | 20.8 |
| 22 | 3 | 8 | 0.0 | 0.0 | 4.2 | 4.2 | 12.5 |
| 23 | 3 | 3 | 0.0 | 0.0 | 8.3 | 8.3 | 20.8 |
| 24 | 3 | 1 | 16.7 | 29.2 | 37.5 | 37.5 | 50.0 |
| 25 | 4 | 2 | 12.5 | 41.7 | 45.8 | 54.2 | 66.7 |
| 26 | 4 | 6 | 29.2 | 45.8 | 54.2 | 58.3 | 62.5 |
| 27 | 4 | 1 | 8.3 | 37.5 | 50.0 | 58.3 | 66.7 |
| 28 | 4 | 3 | 0.0 | 0.0 | 0.0 | 0.0 | 12.5 |
| 29 | 4 | 5 | 12.5 | 25.0 | 41.7 | 41.7 | 58.3 |
| 30 | 4 | 4 | 4.2 | 12.5 | 12.5 | 16.7 | 37.5 |
| 31 | 4 | 7 | 0.0 | 4.2 | 8.3 | 16.7 | 54.2 |
| 32 | 4 | 8 | 4.2 | 20.8 | 25.0 | 33.3 | 45.8 |

- Efficacy trial – Pythium incidence (%)

| | | | 02.04.2021 | 05.04.2021 | 07.04.2021 | 12.04.2021 | 16.04.2021 |
|---|---|---|---|---|---|---|---|
| **Plot** | **Block** | **Treatment** | **02-Apr** | **05-Apr** | **07-Apr** | **12-Apr** | **16-Apr** |
| 1 | 1 | 4 | 0.0 | 4.2 | 8.3 | 8.3 | 12.5 |
| 2 | 1 | 1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 3 | 1 | 8 | 0.0 | 0.0 | 4.2 | 4.2 | 4.2 |
| 4 | 1 | 6 | 0.0 | 0.0 | 4.2 | 4.2 | 4.2 |
| 5 | 1 | 3 | 0.0 | 0.0 | 0.0 | 0.0 | 8.3 |
| 6 | 1 | 5 | 0.0 | 4.2 | 12.5 | 12.5 | 12.5 |
| 7 | 1 | 2 | 0.0 | 8.3 | 8.3 | 8.3 | 8.3 |
| 8 | 1 | 7 | 0.0 | 4.2 | 4.2 | 4.2 | 4.2 |
| 9 | 2 | 4 | 0.0 | 4.2 | 4.2 | 8.3 | 8.3 |
| 10 | 2 | 8 | 0.0 | 0.0 | 8.3 | 8.3 | 8.3 |
| 11 | 2 | 3 | 0.0 | 0.0 | 0.0 | 0.0 | 8.3 |
| 12 | 2 | 1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 13 | 2 | 2 | 0.0 | 0.0 | 4.2 | 4.2 | 4.2 |
| 14 | 2 | 6 | 0.0 | 0.0 | 8.3 | 8.3 | 8.3 |
| 15 | 2 | 7 | 0.0 | 0.0 | 4.2 | 8.3 | 8.3 |
| 16 | 2 | 5 | 0.0 | 0.0 | 8.3 | 8.3 | 8.3 |
| 17 | 3 | 7 | 0.0 | 0.0 | 0.0 | 0.0 | 8.3 |
| 18 | 3 | 5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 19 | 3 | 4 | 0.0 | 0.0 | 4.2 | 4.2 | 4.2 |
| 20 | 3 | 2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 21 | 3 | 6 | 0.0 | 4.2 | 4.2 | 4.2 | 4.2 |
| 22 | 3 | 8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 23 | 3 | 3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 24 | 3 | 1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 25 | 4 | 2 | 0.0 | 4.2 | 8.3 | 8.3 | 16.7 |
| 26 | 4 | 6 | 0.0 | 0.0 | 12.5 | 12.5 | 33.3 |
| 27 | 4 | 1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 28 | 4 | 3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 29 | 4 | 5 | 0.0 | 4.2 | 4.2 | 4.2 | 20.8 |
| 30 | 4 | 4 | 0.0 | 0.0 | 4.2 | 4.2 | 4.2 |
| 31 | 4 | 7 | 0.0 | 0.0 | 0.0 | 0.0 | 29.2 |
| 32 | 4 | 8 | 0.0 | 0.0 | 0.0 | 0.0 | 4.2 |

- Efficacy trial – Pythium severity (%)

| | | | 02.04.2021 | 05.04.2021 | 07.04.2021 | 12.04.2021 | 16.04.2021 |
|---|---|---|---|---|---|---|---|
| **Plot** | **Block** | **Treatment** | **02-Apr** | **05-Apr** | **07-Apr** | **12-Apr** | **16-Apr** |
| 1 | 1 | 4 | 0.0 | 10.0 | 20.0 | 10.0 | 88.0 |
| 2 | 1 | 1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 3 | 1 | 8 | 0.0 | 0.0 | 10.0 | 20.0 | 0.0 |
| 4 | 1 | 6 | 0.0 | 0.0 | 10.0 | 10.0 | 0.0 |
| 5 | 1 | 3 | 0.0 | 0.0 | 0.0 | 0.0 | 88.0 |
| 6 | 1 | 5 | 0.0 | 10.0 | 10.0 | 10.0 | 88.0 |
| 7 | 1 | 2 | 0.0 | 20.0 | 20.0 | 20.0 | 88.0 |
| 8 | 1 | 7 | 0.0 | 10.0 | 20.0 | 40.0 | 0.0 |
| 9 | 2 | 4 | 0.0 | 50.0 | 30.0 | 50.0 | 88.0 |
| 10 | 2 | 8 | 0.0 | 0.0 | 10.0 | 10.0 | 12.5 |
| 11 | 2 | 3 | 0.0 | 0.0 | 0.0 | 0.0 | 88.0 |
| 12 | 2 | 1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 13 | 2 | 2 | 0.0 | 0.0 | 10.0 | 10.0 | 88.0 |
| 14 | 2 | 6 | 0.0 | 0.0 | 10.0 | 10.0 | 0.0 |
| 15 | 2 | 7 | 0.0 | 0.0 | 10.0 | 10.0 | 0.0 |
| 16 | 2 | 5 | 0.0 | 0.0 | 15.0 | 15.0 | 88.0 |
| 17 | 3 | 7 | 0.0 | 0.0 | 0.0 | 0.0 | 88.0 |
| 18 | 3 | 5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 19 | 3 | 4 | 0.0 | 0.0 | 30.0 | 30.0 | 0.0 |
| 20 | 3 | 2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 21 | 3 | 6 | 0.0 | 10.0 | 10.0 | 10.0 | 0.0 |
| 22 | 3 | 8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 23 | 3 | 3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 24 | 3 | 1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 25 | 4 | 2 | 0.0 | 10.0 | 15.0 | 15.0 | 88.0 |
| 26 | 4 | 6 | 0.0 | 0.0 | 10.0 | 10.0 | 88.0 |
| 27 | 4 | 1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 28 | 4 | 3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 29 | 4 | 5 | 0.0 | 10.0 | 10.0 | 10.0 | 88.0 |
| 30 | 4 | 4 | 0.0 | 0.0 | 30.0 | 50.0 | 88.0 |
| 31 | 4 | 7 | 0.0 | 0.0 | 0.0 | 0.0 | 88.0 |
| 32 | 4 | 8 | 0.0 | 0.0 | 0.0 | 0.0 | 88.0 |

- Efficacy trial – Seedling vigour (1-5)

| Plot | Block | Treatment | 02.04.2021 | 05.04.2021 | 07.04.2021 | 12.04.2021 | 16.04.2021 |
|---|---|---|---|---|---|---|---|
| | | | 02-Apr | 05-Apr | 07-Apr | 12-Apr | 16-Apr |
| 1 | 1 | 4 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| 2 | 1 | 1 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| 3 | 1 | 8 | 0.0 | 100.0 | 100.0 | 80.0 | 100.0 |
| 4 | 1 | 6 | 0.0 | 100.0 | 100.0 | 90.0 | 100.0 |
| 5 | 1 | 3 | 0.0 | 50.0 | 70.0 | 80.0 | 100.0 |
| 6 | 1 | 5 | 100.0 | 50.0 | 70.0 | 80.0 | 90.0 |
| 7 | 1 | 2 | 100.0 | 80.0 | 90.0 | 90.0 | 100.0 |
| 8 | 1 | 7 | 0.0 | 20.0 | 10.0 | 10.0 | 50.0 |
| 9 | 2 | 4 | 50.0 | 75.0 | 50.0 | 50.0 | 60.0 |
| 10 | 2 | 8 | 100.0 | 100.0 | 100.0 | 90.0 | 80.0 |
| 11 | 2 | 3 | 0.0 | 0.0 | 50.0 | 90.0 | 90.0 |
| 12 | 2 | 1 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| 13 | 2 | 2 | 100.0 | 80.0 | 85.0 | 85.0 | 95.0 |
| 14 | 2 | 6 | 50.0 | 80.0 | 75.0 | 80.0 | 100.0 |
| 15 | 2 | 7 | 75.0 | 60.0 | 70.0 | 90.0 | 100.0 |
| 16 | 2 | 5 | 100.0 | 50.0 | 75.0 | 60.0 | 90.0 |
| 17 | 3 | 7 | 0.0 | 20.0 | 10.0 | 20.0 | 100.0 |
| 18 | 3 | 5 | 0.0 | 20.0 | 20.0 | 60.0 | 100.0 |
| 19 | 3 | 4 | 0.0 | 0.0 | 10.0 | 10.0 | 75.0 |
| 20 | 3 | 2 | 0.0 | 0.0 | 0.0 | 0.0 | 75.0 |
| 21 | 3 | 6 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| 22 | 3 | 8 | 0.0 | 0.0 | 10.0 | 10.0 | 50.0 |
| 23 | 3 | 3 | 0.0 | 0.0 | 10.0 | 10.0 | 50.0 |
| 24 | 3 | 1 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| 25 | 4 | 2 | 75.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| 26 | 4 | 6 | 80.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| 27 | 4 | 1 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| 28 | 4 | 3 | 0.0 | 0.0 | 0.0 | 0.0 | 10.0 |
| 29 | 4 | 5 | 80.0 | 90.0 | 90.0 | 100.0 | 100.0 |
| 30 | 4 | 4 | 90.0 | 60.0 | 50.0 | 50.0 | 75.0 |
| 31 | 4 | 7 | 0.0 | 20.0 | 10.0 | 20.0 | 90.0 |
| 32 | 4 | 8 | 50.0 | 20.0 | 25.0 | 30.0 | 90.0 |

- Efficacy trial – Fresh weights (g)

| Plot | Block | Treatment | 16.04.2021<br>16-Apr |
|---|---|---|---|
| 1 | 1 | 4 | 0.4 |
| 2 | 1 | 1 | 0.1 |
| 3 | 1 | 8 | 0.2 |
| 4 | 1 | 6 | 0.2 |
| 5 | 1 | 3 | 0.1 |
| 6 | 1 | 5 | 0.2 |
| 7 | 1 | 2 | 0.3 |
| 8 | 1 | 7 | 0.0 |
| 9 | 2 | 4 | 0.2 |
| 10 | 2 | 8 | 0.4 |
| 11 | 2 | 3 | 0.0 |
| 12 | 2 | 1 | 0.5 |
| 13 | 2 | 2 | 0.3 |
| 14 | 2 | 6 | 0.4 |
| 15 | 2 | 7 | 0.4 |
| 16 | 2 | 5 | 0.4 |
| 17 | 3 | 7 | 0.3 |
| 18 | 3 | 5 | 0.1 |
| 19 | 3 | 4 | 0.0 |
| 20 | 3 | 2 | 0.0 |
| 21 | 3 | 6 | 0.2 |
| 22 | 3 | 8 | 0.0 |
| 23 | 3 | 3 | 0.1 |
| 24 | 3 | 1 | 0.6 |
| 25 | 4 | 2 | 0.7 |
| 26 | 4 | 6 | 0.9 |
| 27 | 4 | 1 | 0.6 |
| 28 | 4 | 3 | 0.0 |
| 29 | 4 | 5 | 0.6 |
| 30 | 4 | 4 | 0.2 |
| 31 | 4 | 7 | 0.3 |
| 32 | 4 | 8 | 0.4 |

e. Photos

1. Trial set-up

2 Early emergence

**3. Emergence 09 April 2021**

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