SCEPTREPLUS

Final Trial Report

Trial code:	SP26
Title:	Evaluation of new seed treatments for control of seed-borne Septoria in celery
Crop	Field vegetables - celery
Target	Septoria leaf spot (*Septoria apiicola*), SEPTAP
Lead researcher:	Dave Kaye (ADAS)
Organisation:	RSK ADAS Ltd.
Period:	January 2019 - January 2020
Report date:	31st January 2020
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Report authorised by:	Barry Mulholland
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: 5 June 2020 Authors signature: D. Kaye.

Grower Summary

Introduction

The fungal plant pathogen *Septoria apiicola* causes Septoria leaf spot, one of the most destructive diseases of field-grown celery crops. Seed treatment remains an important component of disease management for celery Septoria, with seed-borne inoculum considered to be the major cause of outbreaks. Effective control of this disease however, has been 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. A trial conducted under controlled environment conditions was designed to identify potential new seed treatments for celery Septoria that could provide an alternative to thiram, for both conventional and organic celery production.

Methods

Efficacy and crop safety experiments were conducted with an untreated batch of celery seed of the susceptible variety Victoria, kindly provided by Tozers Seeds. Although already naturally infested, this seed was artificially inoculated with a spore suspension of *S. apiicola* ($1x\ 10^6$ spores ml^{-1}) to ensure a sufficient and even distribution of inoculum was available for experimental testing. Eight seed treatments were tested, including untreated seed and seed treated with the industry standard product, Agrichem Flowable Thiram (Thiram). Four treatments were applied by Elsoms Seeds Ltd. using commercial seed treatment equipment, whilst two treatments, hydrogen peroxide and Glacial acetic acid (acetic acid) were applied in the Pathology Laboratory at ADAS Boxworth.

Phytotoxicity

The effect of treatments on celery seed germination was assessed after 28 days on filter paper in sealed plastic boxes 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: seed which remain 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.

Seed germination after storage
Samples of celery seed (10 g) from each treatment as well as the untreated sample were stored at ADAS Boxworth for four months representative of medium to long-term commercial storage practices. After this time, seed germination tests were set up as described previously**.**

Seed treatment efficacy
The method chosen for evaluation of efficacy of treatments against *S. apiicola* in celery followed the International Seed Federation guidelines published in February 2019 ‘’Detection of *Septoria apiicola* in Celery and Celeriac seed’’, modified to take into account a smaller batch of seed. Spore suspensions of *S. apiicola* were prepared by soaking and agitating each of the treated seed batches in 50 ml of sterile distilled water (SDW) for 30 minutes. For each of the treated batches, 20 µl droplets were removed from the spore solutions and used to inoculate three separate leaves from young celery plants. The inoculated leaves for each treatment were assessed at 14, 21 and 28 days after inoculation for the presence of visible lesions on leaves (incidence), and the proportion the leaf area displaying symptoms (% severity). Infection was additionally confirmed using microscopy to confirm *S. apiicola* pycnidia presence.

Results

Phytotoxicity

Treatment differences were observed for germination rate and the quality of germinated seedlings (Table 1, Figure 1). Hydrogen peroxide was the only test treatment which did not significantly reduce ($p<0.05$) normal seed germination at this time when compared with both the untreated control and industry standard treatment Agrichem Flowable Thiram. AHDB9850 significantly reduced normal seed germination compared with the industry standard (80.2% vs. 94.4%), but to a lesser extent when compared with the other test products. AHDB9849, AHDB9848, acetic acid and AHDB9847 had a severe impact on normal germination reducing rates to 26.2%, 18.0%, 4.6% and 0.0% respectively. An increase in weak and abnormal seed development was also observed in seed treated with Agrichem Flowable Thiram.

Of the seed which did not germinate, seed death was greatest in seed treated with AHDB9850, AHDB9849, AHDB9848, acetic acid and AHDB9847 compared with the untreated. The number of dead seeds treated with AHDB9850 however was not statistically different ($p<0.05$) compared with seed treated with the standard, Agrichem Flowable Thiram.

Table 1. Effect of plant protection products and basic substances on germination of celery seed on filter paper (n= 50 seeds, 10 boxes per treatment)

Treatment	Mean number seeds germinated / 50			Mean number of seeds not germinated / 50	
	Normal	Weak	Abnormal	Viable seed	Dead seed
Untreated	47.2	**1.5**	**0.0**	1.0	0.3
Agrichem Flowable Thiram	44.1	4.5	0.2	0.6	0.3
AHDB9850	**40.1**	**2.2**	**0.0**	**5.6**	2.1
AHDB9849	**13.1**	**0.0**	**0.0**	**30.8**	**6.1**
Hydrogen peroxide	46.5	3.0	**0.0**	0.5	0.0
Acetic acid	**2.3**	**0.0**	**0.0**	**37.3**	**10.4**
AHDB9848	**9.0**	**0.0**	**0.0**	**23.8**	**17.2**
AHDB9847	**0.0**	**0.0**	**0.0**	**34.8**	**15.2**
	Significantly different from untreated control ($p>0.05$)				
	Not significantly different from untreated control ($p>0.05$)				
Bold values	Significantly different from Agrichem Flowable Thiram ($p>0.05$)				

Figure 1. Effect of plant protection products and basic substances, on germination and seedling quality of celery seed, expressed as the percentage total of seeds assessed (n= 500 seeds per treatment), shortly following treatment.
Germinated seed: normal, weak or abnormal. Ungerminated seed: viable (alive), or dead (soft/rotten).

Seed germination after storage

Seed was stored in the ADAS seed store for four months to determine the long term impact of treatments on germination rate and seedling quality (Table 2). This time period was chosen as it is representative of medium to long-term commercial storage practices. Seed was stored the dark, under ambient conditions (not refrigerated).

Agrichem Flowable Thiram treatment continued to have no impact on germination rates compared with the untreated control, but a significant reduction ($p<0.05$) in seed germination was observed in seed treated with hydrogen peroxide. No reduction in the germination rate of hydrogen peroxide treated seed was observed during the germination test shortly after treatment. This suggests an issue with the rate of hydrogen peroxide tested. Similarly the number of weak seedlings developing from seeds treated with hydrogen peroxide significantly increased compared to the untreated control (15.2% vs. 0.8%).

The germination rate of stored seed treated with AHDB9850 remained similar to the rate from the earlier germination test. After four months of storage normal germination of seed treated with AHDB9849 and acetic acid also remained broadly unchanged, but the germination of seed treated with AHDB9848 reduced from 18% to zero. Germination of seed treated with AHDB9847 remained at zero.

Table 2. Effect of plant protection products and basic substances on germination of celery seed on filter paper after four months storage (n= 50 seeds, 10 boxes per treatment)

Treatment	Mean number seeds germinated / 50			Mean number of seeds not germinated / 50	
	Normal	Weak	Abnormal	Viable seed	Dead seed
Untreated	47.3	0.4	0.0	2.2	0.1
Agrichem Flowable Thiram	45.7	1.3	0.2	2.4	0.4
AHDB9850	**43.7**	1.1	0.0	4.1	1.1
AHDB9849	**14.9**	0.4	0.2	**25.7**	**8.8**
Hydrogen peroxide	**41.7**	**7.6**	0.0	0.4	0.3
Acetic acid	**1.3**	0.0	0.0	**35.6**	**13.1**
AHDB9848	**0.0**	0.0	0.0	**36.6**	**13.4**
AHDB9847	**0.0**	0.0	0.0	**35.5**	**14.5**
	Significantly different from untreated control (p>0.05)				
	Not significantly different from untreated control (p>0.05)				
Bold values	Significantly different from Agrichem Flowable Thiram (p>0.05)				

Figure 2. Effect of plant protection products and basic substances, on germination and seedling quality of celery seed, expressed as the percentage total of seeds assessed (n= 500 seeds per treatment) after four months of storage.
Germinated seed: normal, weak or abnormal. Ungerminated seed: viable (alive), or dead (soft/rotten).

Seed treatment efficacy

Inoculating young (disease free) celery plants with spore solutions from artificially inoculated and treated seed revealed differences in the efficacy of the eight treatments. All treatments significantly ($p<0.05$) reduced the incidence and severity of celery Septoria at one or more assessments. Seed treated with AHDB9850 did not significantly reduce Septoria incidence compared with the untreated control at 14 days post inoculation (dpi), but all other treatments did. At 21 dpi, all treatments significantly reduced septoria incidence ($p>0.01$). No product reduced Septoria incidence at 28 dpi ($p=0.051$), including Agrichem Flowable Thiram.

All products gave significant control of disease severity at 14, 21 and 28 dpi, giving between 71% control (AHDB9847) and 83% control (Agrichem Flowable Thiram and acetic acid) by 28 dpi (Figure 3). No products trialed gave improved control ($p<0.05$) compared with Agrichem Flowable Thiram.

Table 3. Effect of plant protection products and basic substances on mean % incidence and severity of celery septoria after inoculation of celery plants with seed washings at three assessment dates.

Treatment	% disease					
	02/12/19 14 dpi		09/12/19 21 dpi		02/12/19 28 dpi	
	Incidence	Severity	Incidence	Severity	Incidence	Severity
Untreated	75.00	4.33	100.00	10.21	100.00	28.96
Agrichem Flowable Thiram	41.67	1.75	45.83	2.71	62.50	5.00
AHDB9850	58.33	2.63	41.67	4.17	87.50	7.08
AHDB9849	25.00	1.17	41.67	2.29	79.17	8.33
Hydrogen peroxide	29.17	1.67	50.00	3.33	75.00	7.71
Acetic acid	41.67	2.00	41.67	2.29	58.33	5.00
AHDB9848	20.83	1.04	54.17	3.13	75.00	6.25
AHDB9847	45.83	2.50	41.67	3.13	83.33	7.92
	Significantly different from untreated control (p>0.05)					
	Not significantly different from untreated control (p>0.05)					

Figure 3. Effect of plant protection products and basic substances on mean % severity of leaf spot following inoculation of plants with seed washings at 28 dpi.

Conclusions

Germination:

- Treatment with Agrichem Flowable Thiram and hydrogen peroxide did not reduce normal germination rates compared with the untreated control in tests carried out shortly after seed treatment application.
- After four months of storage the germination rate of seed treated with hydrogen peroxide was significantly lower than seed treated with Agrichem Flowable Thiram and the untreated seed (90.4% vs. 83.4% and 94.6% respectively).
- A significant reduction ($p<0.05$) in normal seed germination was observed in seed treated with AHDB9850 compared with both the untreated and Agrichem Flowable Thiram immediately after treatment. The number of weak seedlings was significantly lower in seed treated with AHDB9850 compared with Agrichem Flowable Thiram at this time.

- AHDB9847, AHDB9848, AHDB9849 and acetic acid all severely reduced normal germination immediately after treatment and continued to do so after four months of storage with no germination in seed treated with AHDB9847 and AHDB9848 at this time.

Efficacy

- Spore solutions from seed washings of treated and artificially inoculated celery seed was used to successfully inoculate young disease free celery plants.
- Spore solutions from all test treatments (apart from AHDB9850 at 14 dpi) significantly reduced ($p<0.05$) the incidence of celery Septoria on young plants at 14 and 21 dpi, compared with the untreated control, but no test product reduced disease incidence at 28 dpi, including Agrichem Flowable Thiram.
- Spore solutions from all seed treatments significantly reduced ($p<0.05$) Septoria symptom severity at 14, 21 and 28 dpi compared with the untreated control.
- No product outperformed Agrichem Flowable Thiram in reducing celery Septoria incidence or severity.

Overall

- Seed treatment with hydrogen peroxide significantly reduced celery Septoria on young plants following leaf inoculation, without impacting seed germination rate shortly after treatment, but treatment slightly reduce germination after four months of storage (83.4% vs. 94.6% in untreated seed).
- Seed treatment with AHDB9850 significantly reduced celery Septoria on young plants following leaf inoculation, but slightly impacted seed germination rate compared with untreated seed shortly after treatment (80.2% vs 94.4 respectively) and also following four months of storage (87.4% vs. 94.6% respectively).
- Hydrogen peroxide and AHDB9850 are promising alternatives to take forward following the revocation of the industry standard, thiram. However the reduced germination rates of seeds treated with these products suggests a possible issue with the rates used. Further studies could investigate lowering these rates.
- AHDB9849. AHDB9848, AHDB9847 and acetic acid all significantly reduced disease incidence and severity, but severely impacted germination. Further studies may need to consider treatment dose/response using a range of concentrations to extrapolate effective seed treatment rates

Take home message:

In a comparison of treatments, the industry standard (warm water and thiram soak) remains the most effective treatment in reducing celery Septoria without affecting seed germination or vigour. However, encouraging results were obtained with hydrogen peroxide and to a lesser extent AHDB9850, despite small reductions in germination rates. Further work is required to identify effective dose rates for application to commercial practice and as a replacement for current chemical control products.

Summary

The fungal plant pathogen *Septoria apiicola* causes Septoria leaf spot, one of the most destructive diseases of field-grown celery crops. Control of this disease is threatened by the recent loss of thiram which is the main seed treatment available to propagators and seed houses. Non chemical alternatives to thiram would be of major benefit to both organic and conventional producers, given continued consumer and retailer pressure for a reduction in use of chemical fungicides.

This two year study investigated potential new foliar applied products and seed treatments for control of Septoria leaf spot for use in both organic and conventionally produced celery. The research consisted of two trials; the first trial in 2018 evaluated foliar applied conventional chemical fungicide and biofungicide products for management of Septoria in the field. The second trial in 2019, as presented here, investigated conventional chemical fungicides, a biopesticide and two basic substances for control of seed borne *S. apiicola*. The trial, conducted under controlled environment conditions identified potential new seed treatments for celery Septoria that could provide an alternative to thiram, for both conventional and organic celery production.

Objectives

1. To evaluate a number of fungicides, biopesticides and basic substances as potential seed treatments for efficacy against celery leaf spot (*S. apiicola*) compared to a commercial standard (thiram).
2. To assess their crop safety in celery.

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

For evaluation of efficacy, the trial followed the guidelines of the ISTA standard published in February 2019 ‘’Detection of Septoria apiicola in Celery and Celeriac seed’’, modified to take into account a smaller batch of seed.

Test site

Item	**Details**
Location address	Boxworth, Cambridge CB23 4NN
Crop	Celery
Cultivar	Victoria
Soil or substrate type	N/A
Agronomic practice	N/A
Prior history of site	N/A

Trial design

Germination tests

Two germination tests were established in the controlled environment cabinets at ADAS Boxworth to determine the germination rates of untreated seed and seed treated with the test products. Germination was assessed shortly after seed treatment was applied and again after the seed had been stored for four months under ambient conditions (dark, not refrigerated). This time period was chosen as it is representative of medium to long-term commercial seed storage practices.

Item	Details
Trial design:	Randomised
Number of replicates:	10
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:	500
Leaf Wall Area Calculations:	N/A

Efficacy trial

Seed was artificially inoculated using spore solutions washed from each treated seed batch which had been earlier inoculated with *S. apiicola*. Three leaves from eight plants were inoculated per treatment and assessed for development of Septoria symptoms.

Item	Details
Trial design	Randomised
Number of replicates	8
Number of plants per plot:	1
Number of leaves per plot:	3
Number of leaves per treatment:	24

Treatment details

AHDB Code	Active substance	Product name or manufacturers code	Formulation batch number	Content of active substance in product	Formulation type[1]
N/A	N/A	Untreated	N/A	N/A	N/A
N/A	Thiram	Agrichem Flowable Thiram	E62353	600 g L^{-1}	Flowable concentrate
AHDB9850	N/D	N/D	N/D	N/D	N/D
AHDB9849	N/D	N/D	N/D	N/D	N/D
N/A	Hydrogen peroxide	Hydrogen peroxide	SLBJ8419V	30 wt. % in H_20	Liquid
N/A	Glacial acetic acid	Acetic acid	09J230517	≤ 99%	Liquid
AHDB9848	N/D	N/D	N/D	N/D	N/D
AHDB9847	N/D	N/D	N/D	N/D	N/D

Methods, assessment and records

Approximately 1 kg of an untreated celery seed of a susceptible variety, Victoria (batch number 03727205, 1000 seed weight – 0.34 g) was obtained from Tozers Seeds. Although already naturally infested, this seed was artificially inoculated with a spore suspension of *S. apiicola* (1x 10^6 spores ml^{-1}) to ensure an even distribution of inoculum for consistent treatment effects to be seen.

Application details

Seed inoculation

A conidial suspension of *S. apiicola* was prepared by growing cultures on PDA for 7 days (20°C, 16:8 hour light:dark cycle). The surface of the colonies were flooding with sterile distilled water (SDW) followed by gentle rubbing of the surface using a sterile bent glass rod, producing a spore suspension. This suspension was filtered through four layers of muslin cloth and adjusted to 1x10^6 conidia ml^{-1} using a hemocytometer. The celery seed batch was divided into 80 g subsamples disinfected by soaking in 1% sodium hypochlorite solution for 1 min, followed by rinsing with SDW three times. The seeds were gently rubbed and dried on the benchtop for 1 hour, sitting on blue tissue paper (one layer under, one layer over to remove excess moisture). The seed subsamples were soaked in 200 ml of the *S. apiicola* spore suspension for 4 hours, after which they were placed on three layers of paper towels and air-dried in a laminar flow cabinet at ambient temperature (~20°C) for 3 days, and stored in new paper bags. Seed treatments took place one week after inoculation was completed.

Seed treatment – Elsoms Seeds Ltd.

Agrichem Flowable Thiram, AHDB9850, AHDB9849, AHDB9848 and AHDB9847 were applied to the celery seed at Elsoms 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.

Seed treatment – RSK ADAS Ltd.

Hydrogen peroxide and acetic acid application to celery seed was carried out in the Pathology Laboratory at ADAS Boxworth. Hydrogen peroxide (Sigma Aldrich, product code 216763) and acetic acid (Sigma Aldrich, product code A6283). 80 g seed samples were tied loosely in muslin and immersed into 1 litre glass beakers containing 500 ml of either a 3% solution of hydrogen peroxide or a 3% solution of acetic acid for 3 hours at ambient temperature (20°C). During treatment, the seed samples were gently agitated every 30 minutes. After soaking in the treatments, the seed samples were drained, and then placed on three layers of paper towels and air-dried in a laminar flow cabinet at ambient temperature for two days.

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	Agrichem Flowable Thiram	N/A, seed treatment	1 L per 300 L water as a soak	A
3	AHDB9850	N/A, seed treatment	100 ml per 100 kg seed	A
4	AHDB9849	N/A, seed treatment	160 ml per 100 kg seed	A

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
5	Hydrogen peroxide	N/A, seed treatment	3% solution	A
6	Acetic acid	N/A, seed treatment	3% solution	A
7	AHDB9848	N/A, seed treatment	1 L per 100 kg seed	A
8	AHDB9847	N/A, seed treatment	100 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
Septoria leaf spot	*Septoria apiicola*	SEPTAP	Present	0% incidence	43% incidence on inoculated leaf material

Assessment details

Phytotoxicity

After seeds had been treated, seed germination tests were set up. Any 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

For each treated seed batch, five random samples weighing 1 g were taken. Fifty seeds from each 1 g sample were placed in plastic boxes containing two pieces of 17.5 x 11.5 x 2.0 cm, pleated filter paper, moistened with SDW. The boxes were incubated at 20°C, 16:8 hour light:dark cycle for 28 days. The boxes were checked every 2-3 days to ensure the filter paper remained moist. There were five replicate boxes per treatment and the experiment was performed twice with a one week interval in between.

Seed germination was 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.

Seed germination after storage

A 10 g sample of seed from each treatment, and a 10 g sample from the untreated sample were enclosed in two paper bags, which in turn were placed into a sandwich box containing sachets of silica gel. The sandwich box and its contents were then sealed using parcel tape. The box was stored in the seed store at ADAS Boxworth for four months under ambient conditions (01/09/2019 to 03/01/2020). After which time seed germination tests were set up as described previously using germination boxes.

Seed treatment efficacy

Quantifying viable *S. apiicola* on treated seed

Petri dish method

For each treated seed batch, three random seed samples weighing 8 g were placed in 30 ml SDW in a 50 ml Falcon tube and tubes placed onto an orbital shaker for 2 hours. The resulting liquid was pipetted into a new Falcon tube and centrifuged at 2000 rpm for 10 min. The supernatant was discarded and the pellet re-suspended in 250 µl of SDW. 50 µl of the resulting spore suspension was spread onto five plates of PDA amended with 20 µg/ml chlortetracycline. The plates were incubated at 20°C for 20 hours (16:8 hour light:dark cycle) after which, the plates were examined microscopically for evidence of spore germination.

Leaf inoculation method

Visual inspection of spore germination on PDA proved too difficult to quantify. Therefore a pathogenicity assay using leaf inoculation was set up, which followed the International Seed Federation guidelines published in February 2019 ''Detection of *Septoria apiicola* in Celery and Celeriac seed'', modified to take into account a smaller batch of seed.

Celery plants were grown to the fifth true leaf stage to provide leaf material with eight plants assigned to each treatment. Spore suspensions of *S. apiicola* from each of the treated seed batches were prepared using the centrifuge-based method described in the previous section, but with an added 5 µl of Tween20 to aid inoculation of leaf material. Three leaves from each plant were tagged with a permanent marker, then inoculated with a 20 µl droplet of spore suspension via pipette. The plants were then bagged for four days to maintain high humidity, and placed in a plant growth chamber set to 21 ± 1°C. Once the bags were removed, plants were watered overhead by hand once a day from above to maintain humidity and encourage spore splash between leaves. The plants were assessed for celery Septoria disease at 14, 21 and 28 days after inoculation (dpi). Incidence was recorded for each of the three inoculated leaves per plant (presence or absence of lesions) and severity as the proportion (%) of total leaf area exhibiting *S. apiicola* symptoms.

Evaluation date	Evaluation Timing (DA)[1]	Growth Stage (BBCH)	Evaluation type	What was assessed and how
07/09/2019	1	N/A	Efficacy	Plates observed for fungal growth (20 HAP)
08/09/2019	2	N/A	Efficacy	Plates observed for fungal growth
09/09/2019	1	N/A	Efficacy	Plates observed for fungal growth (20 HAP)
10/09/2019	2	N/A	Efficacy	Plates observed for fungal growth
30/09/2019	28	N/A	Phytotoxicity	Germination
04/10/2019	28	N/A	Phytotoxicity	Germination
02/12/2019	14	N/A	Efficacy	Disease incidence and severity
09/12/2019	21	N/A	Efficacy	Disease incidence and severity
16/12/2019	28	N/A	Efficacy	Disease incidence and severity
31/01/2020	28	N/A	Phytotoxicity	Germination after long term storage

[1] DA – days after the experiment was begun
[2] HAP – hours after plating

Statistical analysis

Germination trials

The germination tests and efficacy trial were laid out as a randomised complete block design. Statistical analysis was carried using ANOVA with a Duncan's Multiple Range Test in Genstat 18. To assess for differences between treatments compared to the untreated control, 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.

Using disease severity data from the final assessment on the 2nd December 2019, the % efficacy of each product was calculated using the following formula:

$$\text{Percentage control} = 1 - \frac{\text{Disease severity of treatment}}{\text{Disease severity of untreated}} \times 100$$

Results

Phytotoxicity: Germination tests

1. Germination immediately after treatment

Differences were seen between the germination rate, and the quality of germinated seedlings, treated with the test products in germination tests performed shortly after treatment (Table 4). This data, shown as a percentage of the total number of seeds assessed is found in Figure 4 below.

Hydrogen peroxide was the only seed treatment tested not to significantly reduce ($p<0.05$) the normal germination rate compared with untreated seed and Agrichem Flowable Thiram with a germination rate of 93% compared with 94.4% and 88.2% respectively. Treatment with AHDB9850 did significantly reduce normal seed germination compared with Agrichem Flowable Thiram (80.2%), but to a lesser extent when compared with the other seed treatments tested. AHDB9849, AHDB9848, acetic acid and AHDB9847 had a severe impact on normal germination reducing rates to 26.2%, 18.0%, 4.6% and 0.0% respectively. A small, but significant increase in weak and abnormal seed development was observed in seed treated with Agrichem Flowable Thiram when compared with the untreated. This increase was not observed in seed treated with hydrogen peroxide.

Of the seed which did not germinate, significantly greater ($p>0.05$) seed death was recorded on seed treated with AHDB9850, AHDB9849, AHDB9848, acetic acid and AHDB9847 compared with the untreated seed. Interestingly no significant difference ($p>0.05$) in the number of dead seeds was seen between seed treated with AHDB9850 and Agrichem Flowable Thiram, but this was seen in all the other treatments. The number of viable seeds which did not germinate was also significantly greater in seed treated with AHDB9849, AHDB9848, acetic acid, AHDB9847 and AHDB9850 than the untreated. Despite the number of ungerminated viable seed treated with AHDB9850 being higher, it was still considerably lower (11.2%) than the proportion recorded for AHDB9849 (61.6%), acetic acid (74.6%), AHDB9848 (47.6%) and AHDB9847 (69.6%).

Although the normal germination rates of AHDB9849 and AHDB9848 were significantly lower than the untreated and Agrichem Flowable Thiram (26.2% and 18.0% vs. 94.4% and 88.2% respectively), of the seed which did germinate, no weak or abnormal seedlings developed. These treatments had a relatively large number of viable, ungerminated seed suggesting a possible issue with the rate used.

Table 4. Effect of plant protection products and basic substances on germination of celery seed, expressed as the average number of seeds per box (n= 50 seeds per box, 10 boxes)

Treatment	Mean number seeds germinated / 50			Mean number of seeds not germinated / 50	
	Normal	Weak	Abnormal	Viable seed	Dead seed
Untreated	47.2	**1.5**	**0.0**	1.0	0.3
Agrichem Flowable Thiram	44.1	4.5	0.2	0.6	0.3
AHDB9850	**40.1**	**2.2**	**0.0**	**5.6**	2.1
AHDB9849	**13.1**	**0.0**	**0.0**	**30.8**	**6.1**
Hydrogen peroxide	46.5	3.0	**0.0**	0.5	0.0
Acetic acid	**2.3**	**0.0**	**0.0**	**37.3**	**10.4**
AHDB9848	**9.0**	**0.0**	**0.0**	**23.8**	**17.2**
AHDB9847	**0.0**	**0.0**	**0.0**	**34.8**	**15.2**
P value	<0.001	<0.001	0.040	<0.001	<0.001
d.f.	72	72	72	72	72
s.e.d.	1.769	0.797	0.067	2.119	1.962
l.s.d.	3.527	1.589	0.133	4.224	3.911
	Significantly different from untreated control (p>0.05)				
	Not significantly different from untreated control (p>0.05)				
Bold values	Significantly different from the standard - Agrichem Flowable Thiram (p>0.05)				

Figure 4: Effect of plant protection products and basic substances, on germination and seedling quality of celery seed, expressed as the percentage total of seeds assessed (n= 500 seeds per treatment), shortly following treatment.
Germinated seed: normal, weak or abnormal. Ungerminated seed: viable (alive), or dead (soft/rotten).

2. Germination after long term storage

Seed was stored for four months in the ADAS Boxworth seed store, under ambient conditions to determine the long term impact of the test treatments on seed germination rate and developing seedling quality (Table 5, Figure 5). Agrichem Flowable Thiram did not reduce germination rates compared with the untreated seed, but a significant reduction ($p<0.05$) in germination was recorded in seed treated with hydrogen peroxide compared with the

untreated seed (83.4% vs. 94.6%). This reduction was not seen in the germination tests which took place shortly after treatment was applied (Table 4) where the normal germination rate of hydrogen peroxide treated seed was 93.0%.

The number of weak seedlings developing from seeds treated with hydrogen peroxide also significantly increased compared to the untreated control and Agrichem Flowable Thiram (15.2% vs. 0.8% and 2.6% respectively).

The germination rate of AHDB9850 treated seed remained similar to the earlier test result, increasing from 80.2% to 89.4%. Normal germination of seed treated with AHDB9849 and acetic acid also remained broadly the same following four months of storage. However, the germination of seed treated with AHDB9848 reduced from 18.0% to zero over this period. Unsurprising, the germination rate of seed treated with AHDB9847 remained zero at both germination tests.

Table 5. Effect of plant protection products and basic substances on germination of celery seed on filter paper after four months storage (n= 50 seeds, 10 boxes per treatment)

Treatment	Mean number seeds germinated / 50			Mean number of seeds not germinated / 50	
	Normal	Weak	Abnormal	Viable seed	Dead seed
Untreated	47.3	0.4	0.0	2.2	0.1
Agrichem Flowable Thiram	45.7	1.3	0.2	2.4	0.4
AHDB9850	**43.7**	1.1	0.0	4.1	1.1
AHDB9849	**14.9**	0.4	0.2	**25.7**	**8.8**
Hydrogen peroxide	**41.7**	**7.6**	0.0	0.4	0.3
Acetic acid	**1.3**	0.0	0.0	**35.6**	**13.1**
AHDB9848	**0.0**	0.0	0.0	**36.6**	**13.4**
AHDB9847	**0.0**	0.0	0.0	**35.5**	**14.5**
P value	<0.001	<0.001	0.265	<0.001	<0.001
d.f.	72	72	72	72	72
s.e.d.	1.186	0.762	0.1148	1.767	1.590
l.s.d.	2.370	1.523	0.2294	3.531	3.178
	Significantly different from untreated control (p>0.05)				
	Not significantly different from untreated control (p>0.05)				
Bold values	Significantly different from Agrichem Flowable Thiram (p>0.05)				

Effect of plant protection products and basic substances, on germination and seedling quality of celery seed, expressed as the percentage total of seeds assessed (n= 500 seeds per treatment) after four months of storage.
Germinated seed: normal, weak or abnormal. Ungerminated seed: viable (alive), or dead (soft/rotten).

Figure 5. Effect of plant protection products and basic substances, on germination and seedling quality of celery seed after four moth of storage, expressed as the percentage total of seeds assessed (n= 500 seeds per treatment).

Seed treatment efficacy

Visual inspection of spore release from spore germination on PDA proved difficult to quantify. Therefore a pathogenicity assay using leaf inoculation was set up, which followed the International Seed Federation guidelines published in February 2019 ‘’Detection of *Septoria apiicola* in Celery and Celeriac seed’’, modified to take into account a smaller batch of seed.

Artificial inoculation of leaves with spore solutions taken from artificially inoculated treated seed revealed differences in the efficacy of test treatments. All treatments significantly ($p<0.05$) reduced the incidence of celery Septoria during at least one assessment. Only seed treated with AHDB9850 did not significantly reduce Septoria incidence compared with the untreated control at 14 dpi, and all seed treatments significantly reduced incidence at 21 dpi ($p<0.01$). No product reduced Septoria incidence at 28 dpi ($p=0.051$), including the standard Agrichem Flowable Thyram.

All seed treatments significantly reduced severity at 14, 21 and 28 dpi, giving between 71% control (AHDB9847) and 83% control (Agrichem Flowable Thiram and acetic acid) by the final assessment (Figure 5). None of the products trialed gave improved control ($p<0.05$) compared with Agrichem Flowable Thiram at any assessment.

Table 6. Effect of plant protection products and basic substances on mean % incidence and severity of leaf spot after inoculation of celery plants with seed washings.

Treatment	% disease					
	02/12/19 14 dpi		09/12/19 21 dpi		02/12/19 28 dpi	
	Incidence	Severity	Incidence	Severity	Incidence	Severity
Untreated	75.00	4.33	100.00	10.21	100.00	28.96
Agrichem Flowable Thiram	41.67	1.75	45.83	2.71	62.50	5.00
AHDB9850	58.33	2.63	41.67	4.17	87.50	7.08
AHDB9849	25.00	1.17	41.67	2.29	79.17	8.33
Hydrogen peroxide	29.17	1.67	50.00	3.33	75.00	7.71
Acetic acid	41.67	2.00	41.67	2.29	58.33	5.00
AHDB9848	20.83	1.04	54.17	3.13	75.00	6.25
AHDB9847	45.83	2.50	41.67	3.13	83.33	7.92
P value	0.003	<0.01	<0.01	<0.01	0.051	<0.01
d.f.	56	56	56	56	56	56
s.e.d.	13.39	0.70	13.67	1.18	12.82	1.83
l.s.d.	26.82	1.41	26.97	2.36	25.68	3.65
	Significantly different from untreated control (p>0.05)					
	Not significantly different from untreated control (p>0.05)					

Figure 5. Effect of plant protection products and basic substances on mean % incidence of leaf spot following inoculation of plants with seed washings at 28 dpi.

Discussion

Two germination tests, one shortly after treatment application and another following four months of storage, determined the impact of seed treatments on both the germination rate and the quality of emerging celery seedlings. A pathogenicity assay using seed washings from artificially inoculated seed treated with the different crop protection products (following a standard protocol issued by International Seed Federation guidelines) was used to assess the viability spores on young celery plants. Levels of celery septoria were established at sufficient and consistent amounts in the seed treatment efficacy trial to enable significant differences between test treatments, the standard Agrichem Flowable Thiram and the untreated control to be seen.

In addition to conventional fungicide treatment, the biological product AHDB9849 and two basic substances, hydrogen peroxide and acetic acid, were tested for their effectiveness in eliminating Septoria from celery seed. Research has shown that acetic based products such as Jet 5 (5% w/w peroxyacetic acid) can also eliminate some seed-borne diseases. Since both hydrogen peroxide and acetic acid break down to naturally occurring products, they have a low environmental impact and leave no residues on treated crops, thus justifying their investigation in this study.

All seed treatments applied to celery seed artificially inoculated with *S. apiicola* consistently reduced disease severity when inoculated as seed spore washings to untreated plants, compared with seed spore washings from untreated seed. Although all products were effective, none outperformed Agrichem Flowable Thiram.

Seed treated with Agrichem Flowable Thiram and hydrogen peroxide performed best overall. Germination tests of seed treated with these products, shortly after treatment, showed no reduction in germination rate compared with the untreated seed, whilst the other products tested gave reductions. A significant reduction in germination rate after four months of long term storage did develop in seed treated with hydrogen peroxide compared to the untreated control (83.4% compared with 94.6%), implying the rate may be too high and a lower rate could be investigated to maintain the long term viability of seed treated with this product. Similarly treatment with AHDB9850 reduced germination rate during both germination tests, but to a lesser degree than the other test treatments, which could potentially be reduced if 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

Results from Parker *et al*., 2007 suggested that a 16 hour pre-soak may have made celery seed more sensitive to heat treatment, resulting in reduced vigour and a pre-soak was not performed in this work. Treatment with acetic acid had a marked effect on germination. Further studies would need to investigate the effect of acetic acid treatment on germination in the presence or absence of this pre-soak using different concentrations of the substance. This result would also need to be investigated on additional seed lots to determine if the findings were specific to this batch.

In a comparison of treatments, the industry standard (warm water and thiram soak) was still the most effective treatment in reducing celery Septoria without affecting seed germination or vigour. However, comparable results were obtained with hydrogen peroxide and to a lesser extent AHDB9850, indicating their promise of these products as future seed treatment options for celery.

Conclusions

Germination:

- Treatment with Agrichem Flowable Thiram and hydrogen peroxide did not reduce normal germination rates compared with the untreated control in tests carried out shortly after seed treatment application.
- After four months of storage the germination rate of seed treated with hydrogen peroxide was significantly lower than seed treated with Agrichem Flowable Thiram and the untreated seed (90.4% vs. 83.4% and 94.6% respectively).
- A significant reduction ($p<0.05$) in normal seed germination was observed in seed treated with AHDB9850 compared with both the untreated and Agrichem Flowable Thiram immediately after treatment. The number of weak seedlings was significantly lower in seed treated with AHDB9850 compared with Agrichem Flowable Thiram at this time.
- AHDB9847, AHDB9848, AHDB9849 and acetic acid all severely reduced normal germination immediately after treatment and continued to do so after four months of storage with no germination in seed treated with AHDB9847 and AHDB9848 at this time.

Efficacy

- Spore solutions from seed washings of treated and artificially inoculated celery seed was used to successfully inoculate young disease free celery plants.
- Spore solutions from all test treatments (apart from AHDB9850 at 14 dpi) significantly reduced ($p<0.05$) the incidence of celery Septoria on young plants at 14 and 21 dpi, compared with the untreated control, but no test product reduced disease incidence at 28 dpi, including Agrichem Flowable Thiram.
- Spore solutions from all seed treatments significantly reduced ($p<0.05$) Septoria symptom severity at 14, 21 and 28 dpi compared with the untreated control.
- No product outperformed Agrichem Flowable Thiram in reducing celery Septoria incidence or severity.

Overall

- Seed treatment with hydrogen peroxide significantly reduced celery Septoria on young plants following leaf inoculation, without impacting seed germination rate shortly after treatment, but treatment slightly reduce germination after four months of storage (83.4% vs. 94.6% in untreated seed).
- Seed treatment with AHDB9850 significantly reduced celery Septoria on young plants following leaf inoculation, but slightly impacted seed germination rate compared with untreated seed shortly after treatment (80.2% vs 94.4 respectively) and also following four months of storage (87.4% vs. 94.6% respectively).
- Hydrogen peroxide and AHDB9850 are promising alternatives to take forward following the revocation of the industry standard, thiram. However the reduced germination rates of seeds treated with these products suggests a possible issue with the rates used. Further studies could investigate lowering these rates.
- AHDB9849. AHDB9848, AHDB9847 and acetic acid all significantly reduced disease incidence and severity, but severely impacted germination. Further studies may need to consider treatment dose/response using a range of concentrations to extrapolate effective seed treatment rates
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References

Green *et al*., 2007. Celery: Evaluation of alternative seed treatments for the control of Septoria apiicola (celery leaf spot). FV 237a Final Report, December 2003. HDC.

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Appendix

a. Crop diary – events related to growing crop

Crop	Cultivar	Treatment date
Celery	Victoria	19/08/2019

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

Date	Event
Pre trial	
15/05/2019	Seeds obtained from Tozers for pre-tests
31/05/2019	Observations on seed for viability of spores begun
18/08/2019	Seeds sent to Elsoms for treatment
19/08/2019	Seeds treated with 3% acetic acid and 3% H_2O_2 at ADAS Boxworth
30/08/2019	Seeds collected from Elsoms
07/09/2019	Droplet experiment set up to observe pycnidia and spore release on seed
08/09/2019	Seeds observed again for spore release
09/09/2019	Seed washing experiment set up and plated onto PDA
10/09/2019	Plates observed for fungal growth, Plated 20 µl and 50 µl onto PDA with chlortetracycline. Plates observed
15/09/2019	Seed washing experiment repeated
Germination tests	
02/09/2019	250 seeds placed in germination boxes - replicate 1
17/09/2019	Germination experiments first observed
30/09/2019	Germination experiments second observed
01/10/2019	Germination experiments on water agar set up. 250 seed per treatment plated out
15/11/2019	Plates first observed
29/10/2019	Plates second observed
07/10/2019	250 seeds placed in germination boxes - replicate 2
22/10/2019	Germination experiments first observed
04/11/2019	Germination experiments second observed
Efficacy tests	
21/08/2019	72 x 2L pots prepared with John Innes No. 1 and sown with 6 celery seeds per pot
25/10/2019	Seed washing experiment 1 (germination on PDA) set up, Seed washing plated out, seeds observed for spore release
26/10/2019	Plates observed for fungal growth
31/10/2019	Seed washing experiment 2 set up, Seed washing plated out
01/11/2019	Plates observed for fungal growth
18/11/2019	Seed washings prepared and plants inoculated
02/12/2019	First symptoms observed, 14 dpi
09/12/2019	21 dpi assessment
16/12/2019	28 dpi assessment
Long term storage tests	
01/09/2019	10g of seed from each treatment placed in seed store
03/01/2020	Germination test set up
31/01/2020	Observations made and results analysed

c. Raw data from assessments

- Phytotoxicity - germination tests following seed treatment

Replicate	Plate	Assessment Date	Replicate 1: 03/09/2019; Replicate 2; 17/09/2019				
		Category of seedling/seed	Emerged seedling			Non emerged seed	
			Normal	Weak	Abnormal	Fresh	Dead
		Treatment Name					
1	1	Untreated	1	1	45	3	0
1	2	Untreated	1	2	47	2	0
1	3	Untreated	1	3	46	4	0
1	4	Untreated	1	4	50	0	0
1	5	Untreated	1	5	45	2	0
2	1	Untreated	2	1	50	0	0
2	2	Untreated	2	2	45	0	0
2	3	Untreated	2	3	48	2	0
2	4	Untreated	2	4	49	1	0
2	5	Untreated	2	5	47	1	0
1	1	Agrichem Flowable Thiram	44	4	0	1	1
1	2	Agrichem Flowable Thiram	50	0	0	0	0
1	3	Agrichem Flowable Thiram	42	5	1	1	1
1	4	Agrichem Flowable Thiram	45	0	0	1	1
1	5	Agrichem Flowable Thiram	48	2	0	0	0
2	1	Agrichem Flowable Thiram	40	10	0	0	0
2	2	Agrichem Flowable Thiram	45	5	0	0	0
2	3	Agrichem Flowable Thiram	39	8	1	2	0
2	4	Agrichem Flowable Thiram	39	10	0	1	0
2	5	Agrichem Flowable Thiram	49	1	0	0	0
1	1	Acetic acid	2	0	0	40	8
1	2	Acetic acid	3	0	0	47	0
1	3	Acetic acid	2	0	0	30	18
1	4	Acetic acid	2	0	0	48	0
1	5	Acetic acid	2	0	0	39	9
2	1	Acetic acid	3	0	0	35	12
2	2	Acetic acid	5	0	0	30	15
2	3	Acetic acid	1	0	0	36	13
2	4	Acetic acid	2	0	0	38	10
2	5	Acetic acid	1	0	0	30	19
1	1	Hydrogen peroxide	45	5	0	0	0
1	2	Hydrogen peroxide	44	4	0	2	0
1	3	Hydrogen peroxide	45	5	0	0	0
1	4	Hydrogen peroxide	50	0	0	0	0
1	5	Hydrogen peroxide	49	1	0	0	0
2	1	Hydrogen peroxide	46	4	0	0	0
2	2	Hydrogen peroxide	50	0	0	0	0
2	3	Hydrogen peroxide	47	3	0	0	0
2	4	Hydrogen peroxide	41	7	0	2	0
2	5	Hydrogen peroxide	48	1	0	1	0
1	1	AHDB9847	0	0	0	40	10
1	2	AHDB9847	0	0	0	28	22
1	3	AHDB9847	0	0	0	31	19
1	4	AHDB9847	0	0	0	45	5
1	5	AHDB9847	0	0	0	39	11
2	1	AHDB9847	0	0	0	40	10
2	2	AHDB9847	0	0	0	38	12
2	3	AHDB9847	0	0	0	33	17
2	4	AHDB9847	0	0	0	30	20
2	5	AHDB9847	0	0	0	24	26
1	1	AHDB9848	0	0	0	40	10
1	2	AHDB9848	0	0	0	28	22
1	3	AHDB9848	0	0	0	31	19

Replicate	Plate	Assessment Date	Replicate 1: 03/09/2019; Replicate 2; 17/09/2019				
		Category of seedling/seed	Emerged seedling			Non emerged seed	
			Normal	Weak	Abnormal	Fresh	Dead
		Treatment Name					
1	4	AHDB9848	0	0	0	45	5
1	5	AHDB9848	0	0	0	39	11
2	1	AHDB9848	0	0	0	40	10
2	2	AHDB9848	0	0	0	38	12
2	3	AHDB9848	0	0	0	33	17
2	4	AHDB9848	0	0	0	30	20
2	5	AHDB9848	0	0	0	24	26
1	1	AHDB9849	15	0	0	30	5
1	2	AHDB9849	10	0	0	40	0
1	3	AHDB9849	14	0	0	26	10
1	4	AHDB9849	9	0	0	40	1
1	5	AHDB9849	5	0	0	35	10
2	1	AHDB9849	5	0	0	35	10
2	2	AHDB9849	21	0	0	29	0
2	3	AHDB9849	20	0	0	23	7
2	4	AHDB9849	7	0	0	30	13
2	5	AHDB9849	25	0	0	20	5
1	1	AHDB9850	42	2	0	4	2
1	2	AHDB9850	43	1	0	5	1
1	3	AHDB9850	40	3	0	5	2
1	4	AHDB9850	39	4	0	3	4
1	5	AHDB9850	41	0	0	8	1
2	1	AHDB9850	36	2	0	10	2
2	2	AHDB9850	35	5	0	7	3
2	3	AHDB9850	35	4	0	7	4
2	4	AHDB9850	50	0	0	0	0
2	5	AHDB9850	40	1	0	7	2

- Phytotoxicity - germination tests after four months of long term storage of treated seed

Replicate	Plate	Assessment Date	Replicate 1: 03/09/2019; Replicate 2; 17/09/2019				
		Category of seedling/seed	Emerged seedling			Non emerged seed	
			Normal	Weak	Abnormal	Fresh	Dead
		Treatment Name					
1	1	Untreated	46	1	0	3	0
1	2	Untreated	43	1	0	6	0
1	3	Untreated	50	0	0	0	0
1	4	Untreated	50	0	0	0	0
1	5	Untreated	43	1	0	6	0
2	1	Untreated	47	0	0	3	0
2	2	Untreated	47	0	0	3	0
2	3	Untreated	50	0	0	0	0
2	4	Untreated	48	0	0	1	1
2	5	Untreated	49	1	0	0	0
1	1	Agrichem Flowable Thiram	48	2	0	0	0
1	2	Agrichem Flowable Thiram	41	3	0	4	2
1	3	Agrichem Flowable Thiram	48	0	0	2	0
1	4	Agrichem Flowable Thiram	44	2	0	4	0
1	5	Agrichem Flowable Thiram	45	1	0	3	1
2	1	Agrichem Flowable Thiram	41	2	0	7	0
2	2	Agrichem Flowable Thiram	42	3	2	3	0
2	3	Agrichem Flowable Thiram	50	0	0	0	0
2	4	Agrichem Flowable Thiram	48	0	0	1	1
2	5	Agrichem Flowable Thiram	50	0	0	0	0
1	1	Acetic acid	2	0	0	40	8
1	2	Acetic acid	1	0	0	40	9
1	3	Acetic acid	3	0	0	36	11
1	4	Acetic acid	0	0	0	32	18
1	5	Acetic acid	2	0	0	37	11
2	1	Acetic acid	1	0	0	34	15
2	2	Acetic acid	1	0	0	35	14
2	3	Acetic acid	1	0	0	39	10
2	4	Acetic acid	2	0	0	32	16
2	5	Acetic acid	0	0	0	31	19
1	1	Hydrogen peroxide	39	11	0	0	0
1	2	Hydrogen peroxide	47	3	0	0	0
1	3	Hydrogen peroxide	40	9	0	0	1
1	4	Hydrogen peroxide	41	8	0	1	0
1	5	Hydrogen peroxide	42	8	0	0	0
2	1	Hydrogen peroxide	40	10	0	0	0
2	2	Hydrogen peroxide	38	10	0	2	0
2	3	Hydrogen peroxide	36	14	0	0	0
2	4	Hydrogen peroxide	44	3	0	1	2
2	5	Hydrogen peroxide	50	0	0	0	0
1	1	AHDB9847	0	0	0	34	16
1	2	AHDB9847	0	0	0	34	16
1	3	AHDB9847	0	0	0	42	8
1	4	AHDB9847	0	0	0	39	11
1	5	AHDB9847	0	0	0	31	19
2	1	AHDB9847	0	0	0	39	11
2	2	AHDB9847	0	0	0	39	11
2	3	AHDB9847	0	0	0	34	16
2	4	AHDB9847	0	0	0	29	21
2	5	AHDB9847	0	0	0	34	16
1	1	AHDB9848	0	0	0	31	19
1	2	AHDB9848	0	0	0	23	27
1	3	AHDB9848	0	0	0	37	13
1	4	AHDB9848	0	0	0	31	19
1	5	AHDB9848	0	0	0	40	10

Replicate	Plate	Assessment Date	Replicate 1: 03/09/2019; Replicate 2; 17/09/2019				
		Category of seedling/seed	Emerged seedling			Non emerged seed	
			Normal	Weak	Abnormal	Fresh	Dead
		Treatment Name					
2	1	AHDB9848	0	0	0	36	14
2	2	AHDB9848	0	0	0	44	6
2	3	AHDB9848	0	0	0	38	12
2	4	AHDB9848	0	0	0	42	8
2	5	AHDB9848	0	0	0	44	6
1	1	AHDB9849	17	0	0	26	7
1	2	AHDB9849	10	2	0	20	18
1	3	AHDB9849	16	1	0	21	12
1	4	AHDB9849	11	0	1	28	10
1	5	AHDB9849	16	0	0	31	3
2	1	AHDB9849	14	1	0	24	11
2	2	AHDB9849	21	0	1	19	9
2	3	AHDB9849	17	0	0	25	8
2	4	AHDB9849	16	0	0	28	6
2	5	AHDB9849	11	0	0	35	4
1	1	AHDB9850	44	1	0	5	0
1	2	AHDB9850	39	5	0	6	0
1	3	AHDB9850	42	0	0	7	1
1	4	AHDB9850	45	0	0	3	2
1	5	AHDB9850	44	1	0	4	1
2	1	AHDB9850	46	3	0	1	0
2	2	AHDB9850	43	1	0	1	5
2	3	AHDB9850	47	0	0	3	0
2	4	AHDB9850	43	0	0	5	2
2	5	AHDB9850	44	0	0	6	0

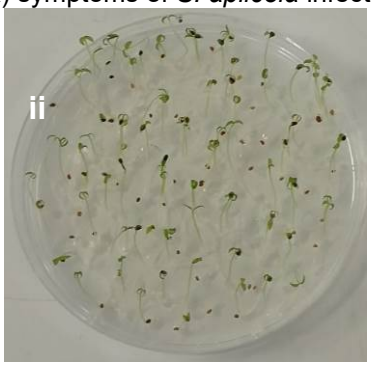
Efficacy trial - seed washing leaf inoculation data

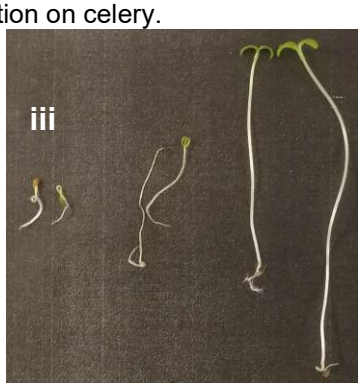
Plant	Assessment Date	02/12/19		09/12/19		16/12/19	
	Day post inoculation	14		21		28	
	Assessment Type	Incidence (%)	Severity (%)	Incidence (%)	Severity (%)	Incidence (%)	Severity (%)
1	Untreated	1.00	8.33	0.33	3.33	1.00	31.70
2	Untreated	0.67	5.00	1.00	11.70	1.00	31.70
3	Untreated	0.67	6.67	1.00	10.00	1.00	33.30
4	Untreated	0.67	5.00	0.67	3.33	1.00	28.30
5	Untreated	0.67	5.00	1.00	11.70	1.00	28.30
6	Untreated	0.67	3.33	0.67	8.33	1.00	40.00
7	Untreated	1.00	8.33	0.67	6.67	1.00	36.70
8	Untreated	0.67	5.00	0.67	11.70	1.00	35.00
1	Agrichem Flowable Thiram	0.00	0.00	0.67	3.33	0.67	10.00
2	Agrichem Flowable Thiram	0.33	1.67	0.33	1.67	0.33	1.67
3	Agrichem Flowable Thiram	0.33	1.00	0.00	0.00	0.67	8.33
4	Agrichem Flowable Thiram	1.00	5.00	0.67	6.67	1.00	16.70
5	Agrichem Flowable Thiram	0.33	1.00	0.33	1.67	0.67	8.33
6	Agrichem Flowable Thiram	0.67	6.67	0.67	3.33	0.67	8.33
7	Agrichem Flowable Thiram	0.33	5.00	0.33	5.00	0.67	5.00
8	Agrichem Flowable Thiram	0.33	1.67	0.67	3.33	0.67	8.33
1	AHDB9850	0.33	1.67	0.33	1.67	1.00	11.70
2	AHDB9850	0.33	1.67	0.33	1.67	1.00	6.67
3	AHDB9850	0.33	5.00	0.67	6.67	1.00	8.33
4	AHDB9850	0.33	5.00	0.33	6.67	1.00	10.00
5	AHDB9850	0.67	6.67	0.00	0.00	0.33	3.33
6	AHDB9850	0.67	6.67	0.67	11.70	0.67	8.33
7	AHDB9850	0.67	6.67	0.67	8.33	0.67	6.67
8	AHDB9850	1.00	10.00	0.33	1.67	1.00	8.33
1	AHDB9850	0.33	1.67	0.33	5.00	0.33	5.00
2	AHDB9849	0.00	0.00	0.33	1.67	0.67	6.67
3	AHDB9849	0.00	0.00	0.33	1.67	0.67	10.00
4	AHDB9849	0.33	1.67	0.33	1.67	0.33	1.67
5	AHDB9849	0.00	0.00	0.67	3.33	0.67	5.00
6	AHDB9849	0.33	5.00	0.33	5.00	0.67	6.67
7	AHDB9849	0.00	0.00	0.33	1.67	0.67	6.67
8	AHDB9849	0.33	1.67	0.00	0.00	0.33	3.33
1	AHDB9849	0.33	1.67	0.67	6.67	1.00	15.00
2	Hydrogen peroxide	0.33	1.67	0.67	8.33	0.67	3.33
3	Hydrogen peroxide	0.33	3.33	0.00	0.00	1.00	13.30
4	Hydrogen peroxide	0.00	0.00	0.33	5.00	0.00	0.00

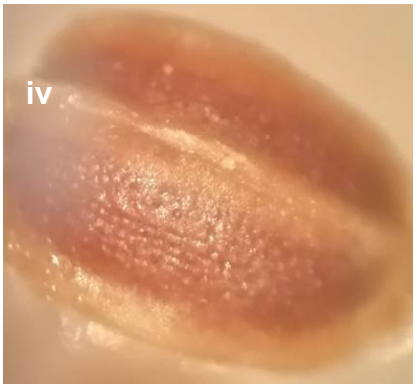
Plant	Assessment Date	02/12/19		09/12/19		16/12/19	
	Day post inoculation	14		21		28	
	Assessment Type	Incidence (%)	Severity (%)	Incidence (%)	Severity (%)	Incidence (%)	Severity (%)
5	Hydrogen peroxide	0.33	1.67	0.67	3.33	0.67	8.33
6	Hydrogen peroxide	0.33	1.67	0.33	1.67	1.00	13.30
7	Hydrogen peroxide	0.00	0.00	0.33	1.67	1.00	10.00
8	Hydrogen peroxide	0.33	1.67	0.67	6.67	0.00	0.00
1	Hydrogen peroxide	0.00	0.00	0.67	3.33	0.67	6.67
2	Acetic acid	0.00	0.00	0.33	1.67	1.00	11.70
3	Acetic acid	0.00	0.00	0.33	1.67	1.00	6.67
4	Acetic acid	0.00	6.67	0.33	6.67	0.67	5.00
5	Acetic acid	0.67	3.33	0.67	1.67	0.33	5.00
6	Acetic acid	0.67	0.00	0.33	0.00	0.67	5.00
7	Acetic acid	0.00	1.67	0.00	6.67	0.33	3.33
8	Acetic acid	0.33	3.33	0.67	1.67	0.33	5.00
1	Acetic acid	0.67	0.00	0.33	6.67	0.33	6.67
2	AHDB9848	0.00	1.67	1.00	6.67	1.00	13.30
3	AHDB9848	0.33	1.67	0.67	0.00	1.00	6.67
4	AHDB9848	0.33	1.67	0.00	0.00	0.67	6.67
5	AHDB9848	0.33	0.00	0.00	1.67	1.00	1.67
6	AHDB9848	0.00	1.67	0.33	6.67	0.33	6.67
7	AHDB9848	0.33	1.67	0.67	1.67	0.67	5.00
8	AHDB9848	0.33	0.00	0.33	1.67	0.33	1.67
1	AHDB9848	0.00	3.33	0.33	8.33	0.33	11.70
2	AHDB9847	0.67	0.00	0.67	5.00	1.00	5.00
3	AHDB9847	0.00	3.33	0.67	0.00	0.67	10.00
4	AHDB9847	0.33	1.67	0.00	5.00	1.00	5.00
5	AHDB9847	0.33	1.67	0.33	3.33	0.67	8.33
6	AHDB9847	0.33	0.00	0.33	1.67	0.67	8.33
7	AHDB9847	0.00	1.67	0.33	1.67	0.67	11.70
8	AHDB9847	0.33	3.33	0.33	6.67	1.00	5.00

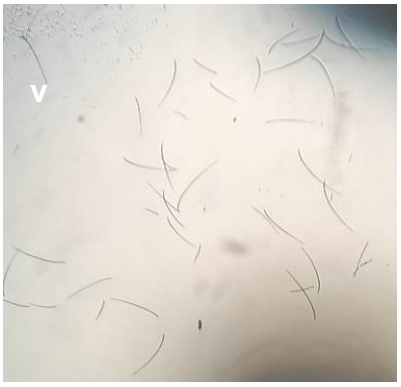
d. photos

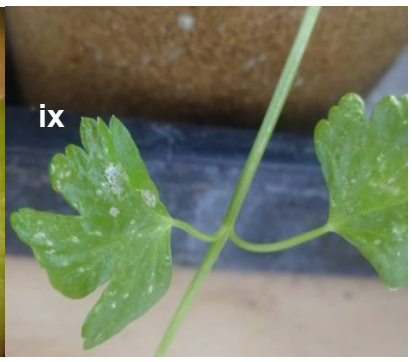
i) Germination boxes set up, ii) germination of untreated seedlings on water agar, ii) classification of seedlings into abnormal, weak and abnormal, iv) pycnidia observed on outside of seed, v) asexual spores of *S. apiicola*, vi) celery plants prior to inoculation, vii) untreated celery plant, viii and ix) symptoms of *S. apiicola* infection on celery.











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