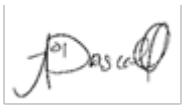
SCEPTREPLUS

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:	Dr Aoife O' Driscoll
Organisation:	RSK ADAS Ltd.
Period:	January 2019 to January 2020
Report date:	31st January 2020
Report author:	Dr Aoife O' Driscoll
ORETO Number: (certificate should be attached)	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



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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 thought to be the major cause of outbreaks of this disease. This has been threatened by the recent loss of thiram; the standard seed treatment against the disease which is applied as a warm water soak. Non chemical alternatives to thiram are relevant to both organic and conventional producers, given continued consumer and retailer pressure for rationalisation of fungicide usage. A 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.

Methods

Efficacy and crop safety experiments were conducted with a batch of celery seed of cv. Victoria which had been confirmed as infected with *S. apiicola*, kindly provided by Tozers Seeds. This was then also reinoculated with a spore suspension of *S. apiicola* at a spore concentration of 1x 10^6 to provide additional assurance of infectivity. Hydrogen peroxide and acetic acid treatment were performed in the Pathology Laboratory at ADAS Boxworth. All other treatments were performed at Elsoms Seeds using standard procedures.

Phytotoxicity

The effect of treatments on germination were assessed quantitatively using germination boxes and on water agar which provided an additional substrate to visualize treatment effects. Seed germination was assessed after 28 days using the following categories:

Normal: Cotyledons at least 50% emerged with no damage to terminal bud. Roots > 1cm.
Weak: Roots 0.5 – 1 cm
Abnormal: Roots <0.5 cm
Ungerminated: Seeds which remain firm and apparently viable at the end of the test
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 and are not fresh.

Seed germination after storage

A 10g sample of seed from each treatment, and a 10g sample from the untreated sample were stored at ADAS Boxworth for four months. After which time seed germination tests were set up as described previously**.**

Efficacy

The method chosen for evaluation of efficacy of treatments against Septoria 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* from each of the treated seed batches were prepared Three leaves from each plant were tagged with a permanent marker, then inoculated with a 20µl droplet of spore suspension. The plants were assessed at 14, 21 and 28 days after inoculation for the presence of pycnidial development.

Results

Phytotoxicity

Table 1: 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	Average number of seeds per box				
	Emerged seedlings			Non emerged seed	
	Normal roots	Weak	Abnormal	Fresh seed	Dead seed
Untreated	47.2	1.5	0	1	0.3
Agrichem Flowable	44.1	4.5	0.2	0.6	0.3

Thiram					
AHDB9850	40.1	2.2	0	5.6	2.1
AHDB9849	6	0	0	30.8	6.1
Hydrogen peroxide	46.5	3	0	0.5	0
Acetic acid	2.3	0	0	37.3	10.4
AHDB9848	11	0	0	34.8	4.2
AHDB9847	0	0	0	34.8	15.2
P value	>0.001	>0.001	0.04	>0.001	>0.001
d.f.	72	72	72	72	72
l.s.d.	3.527	1.59	0.16	4.224	3.911
	Significantly different from untreated control (p>0.05)				
	Not significantly different from untreated control (p>0.05)				

Figure 1: Effect of plant protection products and basic substances, on germination of celery seed, expressed as the percentage total of seeds assessed (n= 500 seeds per treatment).

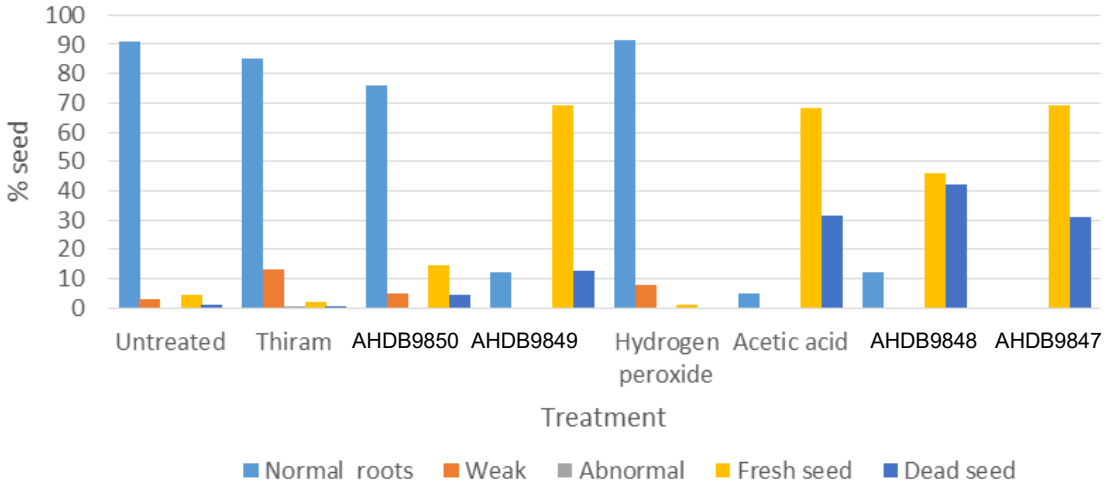
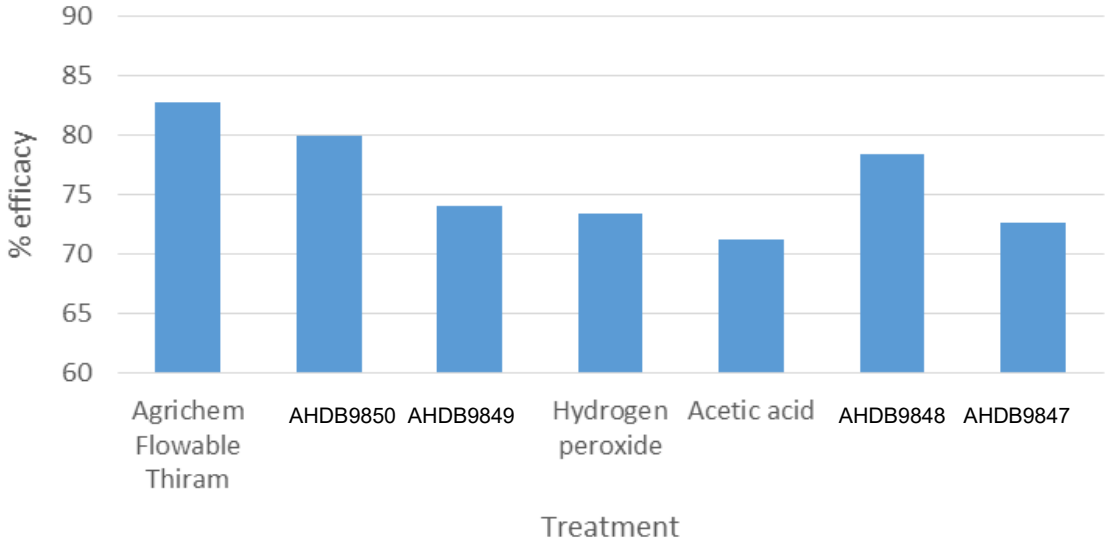


Table 2: Effect of plant protection products and basic substances on mean % incidence of leaf spot following inoculation of celery plants with seed washings.

Treatment	**% disease**					
	02/12/19		**09/12/19**		**02/12/19**	
	14 dpi		**21 dpi**		**28 dpi**	
	Incidence	**Severity**	**Incidence**	**Severity**	**Incidence**	**Severity**
Untreated	5.83	4.33	13.33	10.21	43.12	28.96
Agrichem Flowable Thiram	1.95	1.77	8.01	2.71	18.33	5
AHDB9850	1.62	1.84	9.79	3.17	17.92	5.8
AHDB9849	1.85	1.17	7.5	2.29	13.62	7.5
Hydrogen peroxide	1.66	1.47	9.17	3.33	17.92	7.71
Acetic acid	2.06	2	7.92	2.29	16.04	8.33
AHDB9848	1.94	1.06	8.13	3.12	16.04	6.25
AHDB9847	2.08	2.5	8.96	3.12	18.12	7.92
P value	<0.001	0.752	0.009	<0.001	0.001	<0.001
d.f.	184	184	184	184	184	184
l.s.d.	2.05	1.48	8.12	3.4	13.94	6.67
	Significantly different from untreated control (p>0.05)					
	Not significantly different from untreated control (p>0.05)					

Figure 2: Effect of plant protection products and basic substances on mean % incidence of leaf spot following inoculation of plants with seed washings at three assessment dates.



Conclusions

- AHDB9850 and hydrogen peroxide gave effective pathogen kill without markedly affecting seed germination. These treatments are thus promising to take forward following the revocation of the industry standard, thiram.
- A significant percentage of seed that did germinate following treatment with AHDB9848 and AHDB9849 were classified as having healthy roots suggesting a possible issue with the rate used; further studies could investigate lowering this rate.
- Further studies could investigate the effect of acetic acid, at a lower concentration, in the presence or absence of a pre-soak treatment.

Take home message:

In a comparison of treatments, the industry standard (warm water thiram soak) was still the most effective treatment in terms of pathogen kill without affecting seed vigour. However, very promising results were obtained with AHDB9850 and hydrogen peroxide.

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 has impacted on the number of seed treatments available to propagators and seed houses. Non chemical alternatives to thiram are relevant to both organic and conventional producers, given continued consumer and retailer pressure for rationalisation of fungicide usage.
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, presented herein 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 (*Septoria 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(3)	Phytotoxicity assessment	None
PP 1/181(3)	Conduct and reporting of efficacy evaluation trials including GEP	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
All experimental work was conducted in the Pathology Laboratory or a controlled environment chamber at ADAS Boxworth, with the exception of a number of seed treatments which were applied at Elsoms.

Germination experiments

Item	Details
Trial design:	Replicated, randomised
Number of replicates:	10
Number of seeds per replicate	50

Efficacy trial

Item	Details
Trial design:	Replicated, randomised
Number of replicates:	3 leaves per plant
Number of plants per treatment:	8

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	600g /l	F
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

1 F/FS: Flowable concentrate for seed treatment

Methods, assessment and records

Approximately 1kg of celery seed cv. Victoria (batch number 03727205, 1000 seed weight – 0.34g) was obtained from Tozers Seeds to conduct the trial. This was fresh 2018 harvest seed which was untreated and confirmed infected with *S. apiicola*. Viable spores of *S. apiicola* were detected via real-time PCR using an in-house method developed by Tozers, and incubating followed by microscopic analysis to observe for pycnidial development. To provide additional assurance that the seed was infected, the seed batch was then also inoculated with a spore suspension of *S. apiicola* at ADAS Boxworth.

Application details

Seed inoculation

A conidial suspension of *S. apiicola* was prepared by growing cultures on PDA for 7 days, flooding the surface of the colony with sterile distilled water (SDW) by gently rubbing the surface with a sterile bent glass rod, and filtering the resulting suspension through four layers of cheesecloth. The concentration was adjusted to $1x10^6$ conidia ml^{-1} with a hemocytometer.
The seed batch was divided into 80g subsamples. Each subsample of seed was disinfected by soaking in 1% sodium hypochlorite solution for 1 min and rinsing with SDW three times. The seeds were gently rubbed and then dried on the benchtop for 1 hour, sitting on blue tissue paper (one layer under, one layer over to remove any excess bleach). The seed subsamples were then soaked in 200ml of a spore suspension for 4 hours, placed flat on three layers of paper towels and air-dried in a laminar flow cabinet at ambient temperature (~20°C) for 3 days, then placed in new paper bags. Seed treatment took place one week after drying was completed.

Seed treatment-Elsoms

Five of the products tested in the trial were applied 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 the product required measured using a pipettor. Treatments were applied neat to the seed at the rates required using a pipettor. The product was ejected onto a moving rotary disc which makes the application to the seed 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 mins, or until the seed was at an acceptable relative humidity.

Seed treatment-ADAS

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) were made up to a 3% solution in a laminar flow using SDW. 80g seed samples were tied loosely in muslin and immersed into 1-litre glass beakers containing 500ml 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, then placed flat on three layers of paper towels and air-dried in a laminar flow cabinet at ambient temperature for two days to dry.

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	N/A
2	Agrichem Flowable Thiram	N/A, seed treatment	1l per 300l water as a soak	A
3	AHDB9850	N/A, seed treatment	100ml per 100kg seed	A
4	AHDB9849	N/A, seed treatment	160 ml per 100kg seed	A
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	1L per 100kg seed	A
8	AHDB9847	N/A, seed treatment	100ml per 100kg seed	A

Untreated levels of pests/pathogens at application and through the assessment period

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 and 29% severity on inoculated leaf material

Assessment details

Phytotoxicity

After seeds had been treated, seed germination tests were set up. The effect of treatments on germination were assessed quantitatively using germination boxes and on water agar which provided an additional substrate to visualize treatment effects.

Germination boxes

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

Water agar method

For each seed batch, five random samples weighing 1g were taken. Fifty seeds from each 1g were placed individually on 3% water agar. The petri dishes were sealed with Parafilm and incubated at 20°C (8h light/16h dark) for 28 days. There were five plates per replicate and the experiment was performed once.

Seed germination was assessed after 28 days using the following categories:

Normal: Cotyledons at least 50% emerged with no damage to terminal bud. Roots > 1cm.

- Weak: Roots 0.5 – 1 cm
- Abnormal: Roots <0.5 cm
- Ungerminated fresh seed: Seeds which remain firm and apparently viable at the end of the test
- Dead seed: Seeds which at the end of the test period are either decayed, mouldy or soft or have not produced any seedling or part of a seedling and are not fresh.

Seed germination after storage

A 10g sample of seed from each treatment, and a 10g sample from the untreated sample were enclosed in two paper bags, which in turn was 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. After which time seed germination tests were set up as described previously using germination boxes.

Efficacy

Quantifying viable *S. apiicola* on seed

Visual inspection of seeds

Eight seeds were placed individually onto five plates of PDA (40 seeds in total). A droplet of SDW was added to each seed, then each seed was checked under the microscope for release of spores of *S. apiicola* and a record was made of any seeds with spore release. The plates were incubated for 20 h at 20°C and then re-checked for spore release.

Petri dish method

For each seed batch, three random seed samples weighing 8 g were placed in 30 ml SDW in a 50 ml Falcon tube. The tubes were placed onto an orbital shaker for 2 hours. The resulting liquid was pipetted into a separate Falcon tube and centrifuged at 2000 rpm for 10 min. The supernatant was discarded and the pellet re-suspended in 250 µl of SDW. 50µl of this spore suspension was spread onto each of five plates of PDA amended with 20ug/ml chlortetracycline. The plates were incubated at 20°C for 20 h. After this time, the plates were examine microscopically for evidence of spore germination.

Leaf inoculation method

Visual inspection of spore release from pycnidia and on plate culture proved difficult to quantitiavely measure. Thus 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 for the inoculation assay. There were eight plants per treatment. Spore suspensions of *S. apiicola* from each of the treated seed batches were prepared using the centrifuging method described in the previous section, but with an added 5µl of Tween20 to aid inoculation of leaf material. Three leaves from each plant were tagged with a permanent marker, then inoculated with a 20µl droplet of spore suspension. The plants were then bagged for four days to maintain humidity, with the chamber temperature set to 21 ± 1°C. Once the bags were removed, they were watered by hand once a day from above to maintain humidity and encourage spore splash between leaves. The plants were assessed at 14, 21 and 28 days after inoculation for the presence of pycnidial development.

Evaluation date	Evaluation Timing (DA)*	Crop Growth Stage (BBCH)	Evaluation type (efficacy, phytotox)	What was assessed and how
07/09/2019	1	N/A	Efficacy	Plates observed for fungal growth at 20 hours after plating (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

* DA – days after the experiment was begun

Statistical analysis

The trial was laid out as a randomised complete block design. Statistical analysis was carried using ANOVA with a Duncan’s Multiple Range Test in Genstat 12.2. 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:

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

Results

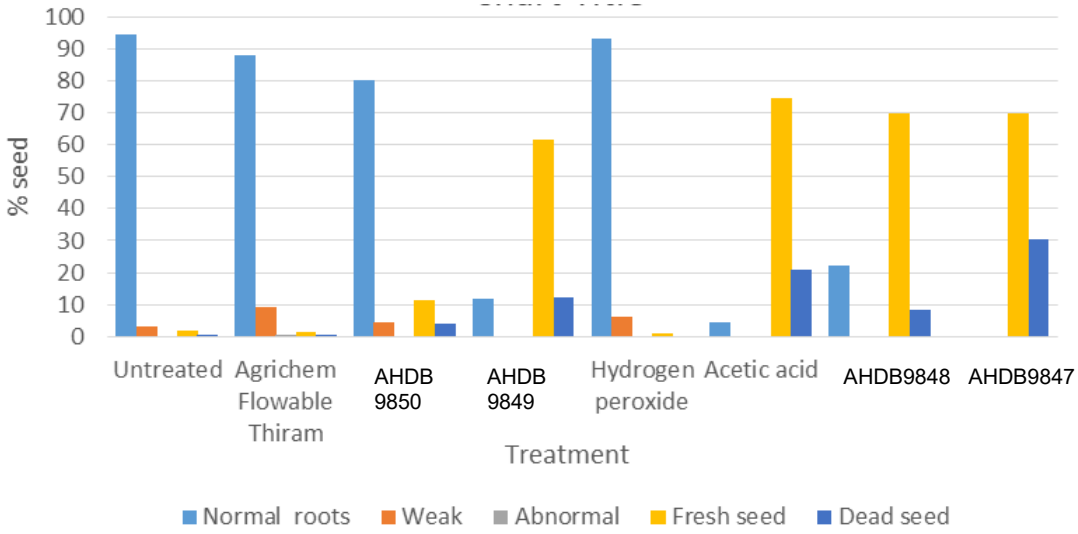
Phytotoxicity

For each treatment, Table 3 shows the number of seeds per germination box which were classed into emerged and non-emerged and their emergence type. Figure 3 expresses this number as a percentage of the total number of seeds assessed in the experiment. 94.4% of seedlings which were untreated exhibited normal germination, indicating that the seed batch during this trial was suitable to perform germination tests with (Figure 3). Treatment with thiram performed overall the best in the trial with on average 44.1 seeds per plate (88.2%) exhibiting normal root growth (Table 3, Figure 1). Treatment with AHDB9850 and hydrogen peroxide also resulted in a significant number of normal seedlings (80.2% and 93% with normal roots, respectively). Though a significant number of seeds did not germinate following treatment with AHDB9848 and AHDB9849 (82% and 73.8% respectively) a significant percentage of seeds that did germinate were classified as having healthy roots (12% and 22% for AHDB9849 and AHDB9848, respectively), suggesting a possible issue with the rate used. AHDB9847 performed the least best out of the products test, with no seed germination observed and 30.4% of seed recorded as dead seed (Figure 3)

Table 3: 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	Average number of seeds per box				
	Emerged seedling			Non emerged seed	
	Normal roots	Weak	Abnormal	Fresh seed	Dead seed
Untreated	47.2	1.5	0	1	0.3
Agrichem Flowable Thiram	44.1	4.5	0.2	0.6	0.3
AHDB9850	40.1	2.2	0	5.6	2.1
AHDB9849	6	0	0	30.8	6.1
Hydrogen peroxide	46.5	3	0	0.5	0
Acetic acid	2.3	0	0	37.3	10.4
AHDB9848	11	0	0	34.8	4.2
AHDB9847	0	0	0	34.8	15.2
P value	>0.001	>0.001	0.04	>0.001	>0.001
d.f.	72	72	72	72	72
l.s.d.	3.527	1.59	0.16	4.224	3.911
	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 germination of celery seed, expressed as the percentage total of seeds assessed (n= 500 seeds per treatment).



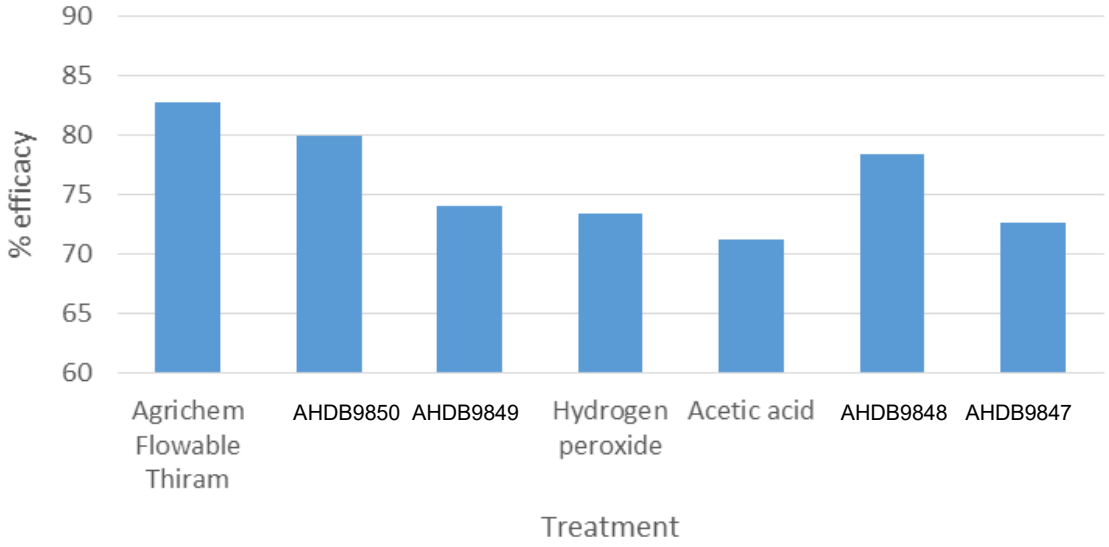
Efficacy

By the final assessment at 21 dpi, washings from seed which had been treated with the standard thiram resulted in an 18.33% incidence and 5% severity, scored as lesions bearing pycnidia on leaf material making it the best performing product in the trial (Table 4). This resulted in an 86% reduction in disease development in comparison to the untreated control (Figure 4). Treatment with AHDB9850 and AHDB9848 resulted in a significant reduction in disease severity by the final assessment (5.8% and 6.25% respectively), giving an overall % control of 80% and 78%, respectively. Percentage control with the remaining products in the trial varied between 71% and 74%

Table 4: Effect of plant protection products and basic substances on mean % incidence and severity of Septoria leaf spot on celery following inoculation of plants with seed washings.

Treatment	**% disease**					
	02/12/19		**09/12/19**		**02/12/19**	
	14 dpi		**21 dpi**		**28 dpi**	
	Incidence	**Severity**	**Incidence**	**Severity**	**Incidence**	**Severity**
Untreated	5.83	4.33	13.33	10.21	43.12	28.96
Agrichem Flowable Thiram	1.95	1.77	8.01	2.71	18.33	5
AHDB9850	1.62	1.84	9.79	3.17	17.92	5.8
AHDB9849	1.85	1.17	7.5	2.29	13.62	7.5
Hydrogen peroxide	1.66	1.47	9.17	3.33	17.92	7.71
Acetic acid	2.06	2	7.92	2.29	16.04	8.33
AHDB9848	1.94	1.06	8.13	3.12	16.04	6.25
AHDB9847	2.08	2.5	8.96	3.12	18.12	7.92
P value	<0.001	0.752	0.009	<0.001	0.001	<0.001
d.f.	184	184	184	184	184	184
l.s.d.	2.05	1.48	8.12	3.4	13.94	6.67
	Significantly different from untreated control (p>0.05)					
	Not significantly different from untreated control (p>0.05)					

Figure 4: Effect of plant protection products and basic substances on % percentage reduction of Septoria leaf spot on celery.



Discussion

Pycnidia of *S. apiicola* were observed microscopically on all of the treated seed batches, however, the number of seeds that showed spore release and spore germination (indicating viability) was sporadic and could not be assessed quantitiavely for experimental purposes. This observation has been made in previous work by both Maude (1996) and Green *et al*., 2007. To overcome this challenge, a pathogenicity assay using seed washings was chosen which followed a standard protocol issued by International Seed Federation guidelines for assessing pathogenicity of spores.

In additional 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. Results from Parker *et al*., 2007 suggested that a 16 hour pre-soak may have made the celery seed more sensitive to heat treatment, resulting in reduced vigour. In this trial, a pre-soak was thus not performed; treatment with hydrogen peroxide had no impact on germination, however 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.

A significant percentage of seeds treated with AHDB9849 and AHDB9848 that did germinate were classified as having healthy roots; this coupled with good efficacy against the pathogen suggest that follow up studies to investigate a lower rate of treatment could be warranted.

Results indicated that treatment with AHDB9850 and hydrogen peroxide gave effective pathogen kill without markedly affecting seed germination, indicating their promise as future seed treatment options for celery.

Conclusions

- AHDB9850 and hydrogen peroxide gave effective pathogen kill without affecting seed germination. These treatments are thus promising to take forward following the revocation of the industry standard, thiram.
- A significant percentage of seed that did germinate following treatment with AHDB9848 and AHDB9849 were classified as having healthy roots suggesting a possible issue with the rate used; further studies could investigate lowering this rate.
- Further studies could investigate the effect of acetic acid, at a lower concentration, in the presence or absence of a pre-soak.

References

- Maude, RB. 1996. Seedborne Diseases and Their Control. Principles and Practice. Wallingford, Oxon: CAB International.
- 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.

Acknowledgements

We would like to thank AHDB and the participating crop protection companies for project funding. Thanks to Tozers Seeds for providing infected seed lots of celery and Elsoms for providing seed treatment facilities.

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 20ul and 50ul 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	Leaf washing experiment 1 (germination on PDA) set up, leaf washing plated out, seeds observed for spore release
26/10/2019	Plates observed for fungal growth
31/10/2019	Leaf washing experiment 2 set up, leaf washing plated out
01/11/2019	Plates observed for fungal growth
18/11/2019	Leaf washings prepared and plants inoculated
02/12/2019	First symptoms observed, 14dpi
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

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	Thiram	44	4	0	1	1
1	2	Thiram	50	0	0	0	0
1	3	Thiram	42	5	1	1	1
1	4	Thiram	45	0	0	1	1
1	5	Thiram	48	2	0	0	0
2	1	Thiram	40	10	0	0	0
2	2	Thiram	45	5	0	0	0
2	3	Thiram	39	8	1	2	0
2	4	Thiram	39	10	0	1	0
2	5	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
1	4	AHDB9848	0	0	0	45	5

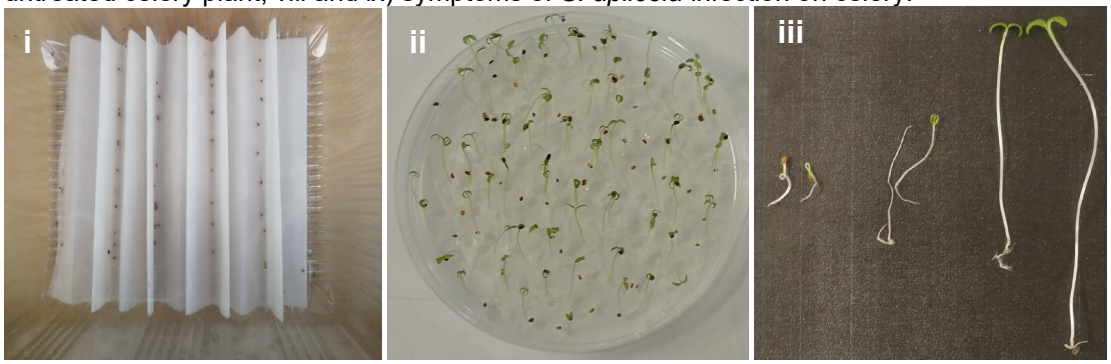
Replicate	Plate	Assessment Date	Replicate 1: 03/09/2019; Replicate 2; 17/09/2019				
		Category of seedling/seed	Emerged seedling			Non emerged seed	
			Normal	Weak	Abnormal	Fresh	Dead
		Treatment Name					
1	5	AHDB9848	0	0	0	39	11
2	1	AHDB9848	0	0	0	40	10
2	2	AHDB9848	0	0	0	38	12
2	3	AHDB9848	0	0	0	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

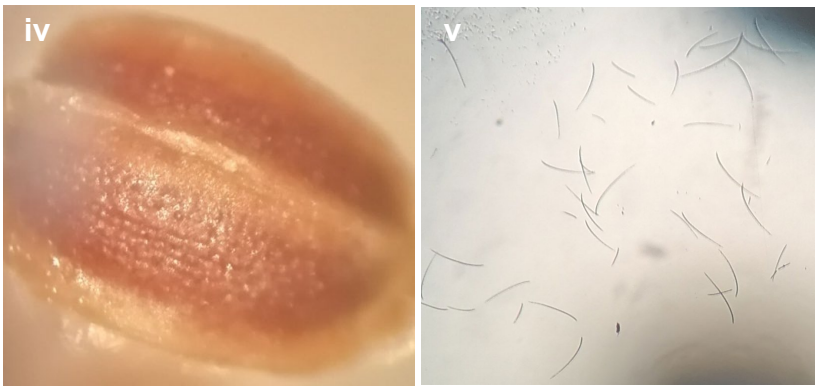
Plant	Assessment Date	02/12/19		09/12/19		16/12/19	
	Day post inoculation	14		21		28	
	Assessment Type	Incidence (%)	Severity (%)	Incidence (%)	Severity (%)	Incidence (%)	Severity (%)
1	Untreated	1	8.33	0.33	3.33	1	31.7
2	Untreated	0.67	5	1	11.7	1	31.7
3	Untreated	0.67	6.67	1	10	1	33.3
4	Untreated	0.67	5	0.67	3.33	1	28.3
5	Untreated	0.67	5	1	11.7	1	28.3
6	Untreated	0.67	3.33	0.67	8.33	1	40
7	Untreated	1	8.33	0.67	6.67	1	36.7
8	Untreated	0.67	5	0.67	11.7	1	35
1	Thiram	0	0	0.67	3.33	0.67	10
2	Thiram	0.33	1.67	0.33	1.67	0.33	1.67
3	Thiram	0.33	1	0	0	0.67	8.33
4	Thiram	1	5	0.67	6.67	1	16.7
5	Thiram	0.33	1	0.33	1.67	0.67	8.33
6	Thiram	0.67	6.67	0.67	3.33	0.67	8.33
7	Thiram	0.33	5	0.33	5	0.67	5
8	Thiram	0.33	1.67	0.67	3.33	0.67	8.33
1	AHDB9850	0.33	1.67	0.33	1.67	1	11.7
2	AHDB9850	0.33	1.67	0.33	1.67	1	6.67
3	AHDB9850	0.33	5	0.67	6.67	1	8.33
4	AHDB9850	0.333	5	0.33	6.67	1	10
5	AHDB9850	0.67	6.67	0	0	0.33	3.33
6	AHDB9850	0.67	6.67	0.67	11.7	0.67	8.33
7	AHDB9850	0.67	6.67	0.67	8.33	0.67	6.67
8	AHDB9850	1	10	0.33	1.67	1	8.33
1	AHDB9850	0.33	1.67	0.33	5	0.33	5
2	AHDB9849	0	0	0.33	1.67	0.67	6.67
3	AHDB9849	0	0	0.33	1.67	0.67	10
4	AHDB9849	0.33	1.67	0.33	1.67	0.33	1.67
5	AHDB9849	0	0	0.67	3.33	0.67	5
6	AHDB9849	0.33	5	0.33	5	0.67	6.67
7	AHDB9849	0	0	0.33	1.67	0.67	6.67
8	AHDB9849	0.33	1.67	0	0	0.33	3.33
1	AHDB9849	0.33	1.67	0.67	6.67	1	15
2	Hydrogen peroxide	0.33	1.67	0.67	8.33	0.67	3.33
3	Hydrogen peroxide	0.33	3.33	0	0	1	13.3
4	Hydrogen peroxide	0	0	0.33	5	0	0
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	13.3

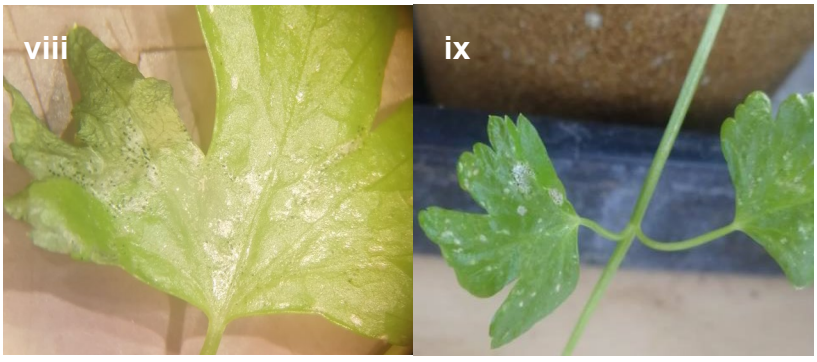
Plant	Assessment Date	02/12/19		09/12/19		16/12/19	
	Day post inoculation	14		21		28	
	Assessment Type	Incidence (%)	Severity (%)	Incidence (%)	Severity (%)	Incidence (%)	Severity (%)
7	Hydrogen peroxide	0	0	0.33	1.67	1	10
8	Hydrogen peroxide	0.33	1.67	0.67	6.67	0	0
1	Hydrogen peroxide	0	0	0.67	3.33	0.67	6.67
2	Acetic acid	0	0	0.33	1.67	1	11.7
3	Acetic acid	0	0	0.33	1.67	1	6.67
4	Acetic acid	0	6.67	0.33	6.67	0.67	5
5	Acetic acid	0.67	3.33	0.67	1.67	0.33	5
6	Acetic acid	0.67	0	0.33	0	0.67	5
7	Acetic acid	0	1.67	0	6.67	0.33	3.33
8	Acetic acid	0.33	3.33	0.67	1.67	0.33	5
1	Acetic acid	0.67	0	0.33	6.67	0.33	6.67
2	AHDB9848	0	1.67	1	6.67	1	13.3
3	AHDB9848	0.33	1.67	0.67	0	1	6.67
4	AHDB9848	0.33	1.67	0	0	0.67	6.67
5	AHDB9848	0.33	0	0	1.67	1	1.67
6	AHDB9848	0	1.67	0.33	6.67	0.33	6.67
7	AHDB9848	0.33	1.67	0.67	1.67	0.67	5
8	AHDB9848	0.33	0	0.33	1.67	0.33	1.67
1	AHDB9848	0	3.33	0.33	8.33	0.33	11.7
2	AHDB9847	0.67	0	0.67	5	1	5
3	AHDB9847	0	3.33	0.67	0	0.67	10
4	AHDB9847	0.33	1.67	0	5	1	5
5	AHDB9847	0.33	1.67	0.33	3.33	0.67	8.33
6	AHDB9847	0.33	0	0.33	1.67	0.67	8.33
7	AHDB9847	0	1.67	0.33	1.67	0.67	11.7
8	AHDB9847	0.33	3.33	0.33	6.67	1	5

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.







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This certifies that

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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:

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

Signature Alison Richardson
Authorised signatory

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