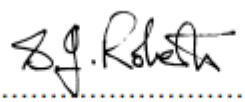
SCEPTREPLUS

Final Trial Report

Trial code:	**SP64**
Title:	**Seed Treatments for Onion Neck Rot**
Crop	**Onions**
Target	**Neck Rot (Botrytis aclada/allii)**
Lead researcher:	**S J Roberts**
Organisation:	**Plant Health Solutions**
Period:	**05/11/2020 to 31/03/2021**
Report date:	**15/03/2021**
Report author:	**S J Roberts**
ORETO Number: (certificate should be attached)	**381 (Warwick Crop Centre)**

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

15/03/2021 .....
Date



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Trial Summary

Introduction

Neck rot can cause significant losses in stored onions. It is mainly caused by two species of *Botrytis: B. aclada, B. allii*. The disease is seed-borne but symptoms are not apparent in the field and only develop in store. Until now most conventional seed has been treated with fungicides to control seed-borne inoculum. With the recent loss of thiram there is a need to identify alternative treatments to minimise seed-borne inoculum and reduce transmission from seed to seedling.

Methods

Fungicide sensitivity plate tests were initially used for preliminary evaluation of chemical fungicides. Onion seed lots were inoculated with different strains of either *B. aclada* or *B. allii.* The inoculated seed was treated in the laboratory, and the efficacy evaluated in two ways: a standard seed test (direct plating on semi-selective agar) and a seed to seedling transmission test.

Results

Table 1. Efficacy of various onion seed treatments on neck rot (*Botrytis aclada/allii*) seed infestation (proportion of seeds upon which the pathogen was detected, and germination (proportion of normal seedlings), and emergence (proportion of seeds sown that emerged) and seed-to-seedling transmission (proportion of seedlings infected). Data represent the combined results of three seed lots, two inoculated with *B. allii*, and one with *B. aclada.*

	Seed tests		**Transmission**		
Treatment	**Infestation (proportion)**	**Germination (proportion)**	**Emergence (proportion)**	**Transmission (proportion)**	**Reduction (percentage)**
Hot water	0.01	0.93	0.95	0.003	99.7
Hydrogen peroxide	1.00	0.86	NT	NT	NT
Acetic acid	0.30	0.93	0.85	0.085	91.5
AHDB9804	0.39	0.94	0.91	0.018	98.2
Maxim 480 FS	0.03	0.90	0.91	0.159	84.1
AHDB9805	0.60	0.93	0.92	0.043	95.7
AHDB9806	0.80	0.95	0.92	0.084	91.6
AHDB9807	0.84	0.90	0.82	1.000	0.0
AHDB9803	0.24	0.40	NT	NT	NT
AHDB9849[B]	NT	NT	0.84	0.997	0.0
AHDB9855[B]	NT	NT	0.83	0.995	0.0
Untreated	1.00	0.94	0.83	0.498	0.0
	Not significantly different from untreated control (p>0.05)				
	Significantly different from untreated control (p<0.05)				

[B] Biological control agent.

Conclusions

- The most effective treatment was hot water (>99% reduction in seed infestation and seed to seedling transmission).
- Significant reductions in disease transmission were achieved with three coded chemical fungicides, they were comparable to the current standard onion seed treatment (Maxim 480 FS), but were not as good as hot water.
- The basic substance vinegar was as effective as the current standard treatment and chemical fungicides.

Take home message:

Hot water was the most effective treatment for control of seed-borne onion neck rot.

Objectives

1. Identify potential seed treatments that may be effective against neck rot.
2. Determine *in vitro* activity of fungicide products against neck rot.
3. Determine the efficacy of physical and fungicide seed treatments on apparent seed infection.
4. Determine the effect of all treatments on seed-to-seedling transmission.
5. Analyse data and prepare report.

Trial conduct

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

Relevant EPPO guideline(s)		**Variation from EPPO**
PP 1/152(4)	Design and analysis of efficacy evaluation trials	None
PP 1/135(3)	Phytotoxicity assessment	None
PP 1/181(4)	Conduct and reporting of efficacy evaluation trials including GEP	None

There were no deviations from EPPO guidance.

Product Details

Table 2. Details of products and treatments examined as potential seed treatments for control of onion neck rot.

AHDB Code	Active substances	Product name	Formulation batch number	Content of active substance in product	Formulation type
HW	na	Hot water			
PE	Hydrogen peroxide	Hydrogen peroxide <5%		4% v/v	Solution
AC	Acetic acid	Vinegar		5% w/v	Solution
AHDB9804	N/D	N/D	N/D		SC
MX	Fludioxonil	Maxim 480 FS	PE-1261SMU8D015	480 g/L	FC
AHDB9805	N/D	N/D	N/D	N/D	SC
AHDB9806	N/D	N/D	N/D	N/D	SC
AHDB9807	N/D	N/D	N/D	N/D	SC
AHDB9803	N/D	N/D	N/D	N/D	na
AHDB9849	N/D	N/D	N/D	N/D	FC
AHDB9955	N/D	N/D	N/D	N/D	WP
UN	na	Untreated			
AHDB9848	N/D	N/D	N/D	N/D	FC
TH	Thiram	Thiram	25099	600 g/L	SC

Table 3. Application rates used for onion seed treatments against neck rot.

No.	Treatment	Code	Active substance	Rate of use (product) (ml/kg)	Timing	Evaluation[1]		
						P	ST	TR
1	Hot water	HW		50°C/30 min	pre-sowing		X	X
2	Hydrogen peroxide	PE	Hydrogen peroxide	40g ai/L (dip in excess 10 min)	pre-sowing		X	
3	Vinegar (food grade)	AC	Acetic acid	50g ai/L (dip in excess 10 min)	pre-sowing		X	X
4	AHDB9804	AHDB9804	N/D	2	pre-sowing		X	X
5	Maxim 480 FS	MX	Fludioxonil	1	pre-sowing	X	X	X
6	AHDB9805	AHDB9805	N/D	1	pre-sowing	X	X	X
7	AHDB9806	AHDB9806	N/D	0.1	pre-sowing	X	X	X
8	AHDB9807	AHDB9807	N/D	2	pre-sowing	X	X	X
9	AHDB9803	AHDB9803	N/D		pre-sowing		X	
10	AHDB9849	AHDB9849	N/D	1.6	pre-sowing			X
11	AHDB9955	AHDB9955	N/D	40	pre-sowing			X
12	Untreated	UN			pre-sowing	X	X	X
	AHDB9848	AHDB9848	N/D	10	pre-sowing	X		
	Thiram	TH	Thiram	5	pre-sowing	X		

[1] P = plate assay, ST = seed test, TR = seed to seedling transmission test.

WP2 Plate Assays

The effect of chemical fungicides on the growth of six pathogen strains were evaluated in plate assays (Table 3).

Test site

Item	Details
Location address	Plant Health Solutions, The Estate Office, Harbury Heath, Leamington Spa, CV33 9NL.
Crop	Onions
Cultivar	Hybrid brown bulb onion

Trial design

Item	Details
Trial design:	Randomised block (confounded with isolate)
Number of isolates	6
Number of replicates:	6 (confounded with isolate)
Number of treatments:	7 (inc. control)
Levels:	3 (Standard, 0.5X, 2X)
Temperature:	21 °C

Isolates

Six isolates were sub-cultured to fresh plates of potato dextrose agar medium (PDA) in advance: three *B. aclada* (8366, 9736, 9752) and three *B. allii* (9722B, 9737, 9931).

Treatment details

Potato dextrose agar (PDA) was prepared according to the manufacturer's instructions, steamed to dissolve and dispensed into 125 mL aliquots. After autoclaving and cooling to 50 °C, fungicide products were added to the aliquots at standard, half and double rates, gently mixed by swirling and inversion, then poured into six 9 cm plates. Plates were allowed to dry/rest at room temperature for 2 d before inoculation. Discs (5 mm) of growth from actively growing cultures of each *Botrytis* isolate were aseptically cut with a sterile cork borer and used to inoculate the centre of each test plate. One plate of each fungicide/concentration combination was inoculated with each isolate. Following inoculation, batches of plates were enclosed in polythene bags to prevent drying out (separate bags for each isolate), and incubated at 21 °C in the dark.

Assessment

The diameter of fungal growth was measured with a ruler at 2 and 5 d after inoculation.

Analysis

Data were analysed using R (R Core Team, 2021), making use of the 'drc' library (Ritz *et al.*, 2015). A three parameter logistic growth model was fitted to the data.

WP3 Seed Treatment and Testing

The effect of physical and chemical fungicide treatments, and basic substances (Table 3) were evaluated for their effect on apparent seed infestation levels by direct plating on a selective medium. This method is not suitable for biological treatments as they are either inhibited by selective agents in the medium or overgrow the plates and mask the presence of the pathogen.

Test site

Item	Details
Location address	Plant Health Solutions, The Estate Office, Harbury Heath, Leamington Spa, CV33 9NL.
Crop	Onions
Cultivar	Hybrid brown bulb onion

Trial design

Item	Details
Trial design:	Randomised block (confounded with isolate)
Number of isolates (~seed lots)	4
Number of treatments:	10 (inc. control)
Number of replicates:	12 plates per treatment (3 per lot/isolate)
Seeds per plate:	25
Temperature:	21 °C

Isolates and seed

A proprietary method was used to inoculate four batches of onion seed. The seed was a standard hybrid brown bulb onion variety. Each batch was inoculated with a

different pathogen isolate: two with *B. aclada* (9736, 9752) and two with *B. allii* (9737, 9931).

Treatment details

Table 4. Details of seed treatments applied to onion seed for potential control of onion neck rot.

Treatment number	Treatment code	Rate of use (product) (ml/kg)	Rate of active substance (ml or g/kg	Date applied
1	HW	50°C/30 min		03/12/20
2	PE	Dip in excess 10 min	40 g/L	04/12/20
3	AC	Dip in excess 10 min	50 g/L	04/12/20
4	AHDB9804	2	0.4, 0.2	04/12/20
5	MX	1	0.48	04/12/20
6	AHDB9805	1	0.025, 0.025	04/12/20
7	AHDB9806	0.1	0.05	04/12/20
8	AHDB9807	2	0.25	04/12/20
9	AHDB9803			09/12/20
10	AHDB9849[B]	1.6	3.5E10 CFU	04/12/20
11	AHDB9955[B]	40	4.0E10 CFU	04/12/20
12	UN			04/12/20

[B] Biological control agent.

Each batch of inoculated seed was divided into 2 g or 2.5 g aliquots in glass screw cap universal bottles. Treatments were applied at room temperature (RT) in the laboratory.

For chemical fungicide treatments, preliminary tests with a coloured product indicated that a treatment volume of 100 µl resulted in even wetting/distribution of product on 2 g of seed, therefore fungicides were diluted in deionised water as appropriate to achieve the required target concentration/volume of product per g of seed. An aliquot of the diluted product was then added to the seeds (100 µl per 2 g of seed) in the bottles and shaken briefly to distribute over the seeds.

One of the biological treatments formulated as a dry powder was added to the seed directly.

For the basic substances (acetic acid and hydrogen peroxide), 20 mL of solution was added to the bottles (i.e. a tenfold excess), agitated, then left to stand for 10 min. Seeds were then drained, blotted dry and spread out in a Petri dish to dry at RT.

For hot water treatment, seeds were immersed in hot water in a water bath for 30 min, then drained, blotted dry and spread out in a Petri dish to dry at RT.

For proprietary physical treatment AHDB9803 aliquots of seed were treated by the technology owner.

Following treatment/drying, the bottles of seed were loosely capped and stored at RT until testing. Post-testing, caps were sealed and seed was stored in the fridge until sown in transmission tests.

Seed testing

Onion seed was tested approx. one week after treatment by direct plating on semi-selective Kritzman's agar medium (Kritzman & Netzer, 1978). Twenty-five seeds were spaced evenly on each 9.0 cm plate. Plates were grouped together (enclosed in polythene bags to prevent drying out) according to the isolate (lot). Plates were then incubated for up to 14 d in the dark at 20-21 °C. Individual seeds on each plate were examined for the presence of typical *B. aclada / B.allii* (*Ba*) morphology (based on sporophores and conidia), using a stereo microscope, after 5-7 days and then again at up 14 days depending on earlier results. The number of infested seeds in each plate was recorded.

An additional 25 seeds for each seed lot x treatment combination were also tested for germination according to the ISTA methods (TP) (ISTA, 2007), in order to assess potential adverse effects of treatments on germination.

Analysis

Analysis was done using R (R Core Team, 2021). Data in the form of the number of infested and healthy seeds in each plate were analysed by fitting a series of Generalised Linear Models using the `glm()` function with a quasi-binomial error distribution, and logistic link function. The seed lot (isolate) and treatment were used as a classifying factors. Means and standard errors for each treatment were obtained as predictions from the appropriate model using the `predict()` function. Germination data were analysed in a similar way.

WP4 Transmission Testing

The most effective treatments and products in the plate and seed treatment assay plus biological treatments (Table 3) were evaluated for their effect on seed-to-seedling transmission.

Test site

Item	Details
Location address	Glasshouse Compartment E8, Warwick Crop Centre, Wellesbourne, Warwick, CV35 9EF
Crop	Onions
Cultivar	Hybrid brown bulb onion
Substrate	Levington F2S (peat based growing medium)
Prior history of site	Used for general plant raising

Trial design

Item	Details
Trial design:	Randomised complete blocks
Number of treatments:	10
Number of replicates:	4 (confounded with isolate/block)
Plot size: (w x l)	6 x 5 = 30 module cells (shallow P60s, half-tray)
Plot size:	1 standard seed tray (25 x 38 cm)
Number of seeds per plot:	210

Environment summary

Table 5. Summary of key environmental parameters in onion neck rot transmission experiment.

Item	Details
Total minutes of irrigation:	54
Total equivalent mm water applied	26.4

Glasshouse min. set temperature (night/day) (°C)	15/18
Vent temperature (°C):	21
Actual Temperature (°C) Mean (Min - Max)	14.8 (11.3 – 25.7)

Experimental details

An automatic (controlled by a solenoid valve linked to the glasshouse computer) overhead sprinkler system was set up on the glasshouse bench. The spacing between sprinkler heads and the water pressure was set to ensure a just overlapping pattern of water delivery. The amount of water delivered by the system was measured by placing two sets of five plant pot saucers across the width of bench, and recording the volume collected in each saucer in one minute.

Glasshouse temperature was set to minimum temperatures of 15/18 °C day/night and venting at 21 °C. Actual temperature was recorded at 30 min intervals by a data logger placed in the centre of the bench.

The same inoculated onion seed was used in this transmission experiment as for direct seed testing, and had been stored in the fridge since testing (38 d).

Half 'P60' shallow module trays were filled with Levington F2S growing medium, levelled off, then lightly compressed. Seven seeds were hand-sown in each cell, with 30 cells (half a tray) per isolate x treatment combination. Seeds were covered with a thin layer of sieved growing medium. Trays were then set out in full seed trays on a glasshouse bench equipped with overhead sprinkler irrigation, and arranged in a randomised block design according to a predefined randomisation plan.

An initial 5 min watering was given shortly after all trays had been set out on the bench, to ensure the growing medium was thoroughly wetted through and settled. Subsequently all trays were watered according to a schedule, adjusted according to perceived need: initially 1 min at 08:00 and 1 min at 15:00 for the first 14 d, then reduced to 1 min at 08:00 for 7 d and then back to twice a day until harvest.

To minimise environmental variability and ensure even watering, trays were rotated one or two positions clockwise within their blocks at 4-5 d intervals.

Assessment details

Phytotoxicity

Seedlings in all plots were observed for signs of phytotoxicity at regular intervals.

Emergence

The number of seedlings in each of the first fifteen cells in each 'plot' were counted 15 d after sowing.

Harvesting and disease assessment

Seedlings were harvested at approximately the first true leaf stage (29 to 30 d after sowing). Seedlings from groups of one, two, or three cells were harvested by cutting off all foliage with scissors just above soil level and transferring to new 15 x 10 cm grip seal polythene bags. Any seeds still adhering to cotyledons were gently removed before seedlings were placed in the bags. For each treatment plot, 3 x 3 cells, 6 x 2 cells, and 6 x 1 cell were harvested (total of 15 groups per plot), for the untreated control plots 15 x 1 cells were harvested. Two blocks were harvested at 29 d and the remaining two at 30 d after sowing. Scissors, hands, surfaces, etc. were disinfected between each plot by spraying/wiping with chlorine solution (0.25%) and/or 70% iso-propanol after each treatment. Scissors were allowed to remain in contact with disinfectant for at least 5 min before wiping dry with paper towel.

Two days after the final harvest, the number of seedling stubs in each cell was counted.

After harvesting, the bags of leaves were transferred to the laboratory and incubated for 7 d in the dark at room temperature. Each bag was then observed under the stereo microscope for the presence of typical *Ba* sporulation on senescent leaf tissues.

Table 6. Details of evaluations in onion neck rot (*Botrytis aclada/allii*) transmission experiment

Evaluation date	Days since sowing	Crop Growth Stage (BBCH)	Evaluation type (efficacy, phytotox)	Assessment
02/02/2021	15	Crook stage, cotyledon still sharply bent (012)	E/P	Emergence (count of number of seedlings in each cell)
24-26/02/2021	37-39	1 TL (100 to 101)	E	Transmission (presence of *Ba* in harvested leaves)

Analysis

Analysis was done using R (R Core Team, 2021). Emergence data were in the form of the number of seedlings in each cell. Data were analysed by fitting a series of Generalised Linear Models using the `glm()` function with a binomial error distribution, and logistic link function. Transmission data were in the form of a binary variable for the presence or absence of *Ba* infection in each bag of harvested leaves. Data were analysed by fitting a series of Generalised Linear Models using the `glm()` function with a binomial error distribution, and complementary-loglog link function. In both cases, the seed lot (isolate), block and treatment were used as a classifying factors. Means and standard errors for each treatment were obtained as predictions from the appropriate model using the `predict()` function.

Results

WP2 Plate Tests

There were clear and statistically significant differences in the effect of the different products on the growth of *Ba* compared to the control (Fig 1.) Differences between the product concentrations were relatively small and not significant, therefore for clarity the combined values are presented. All products reduced the growth rate of *Ba* compared to the untreated control, two products (Maxim 480 FS and AHDB9805) effectively inhibited growth completely (Table 7, Fig 2).

Note that product AHDB9804 was not included in the plate tests as it arrived too late.

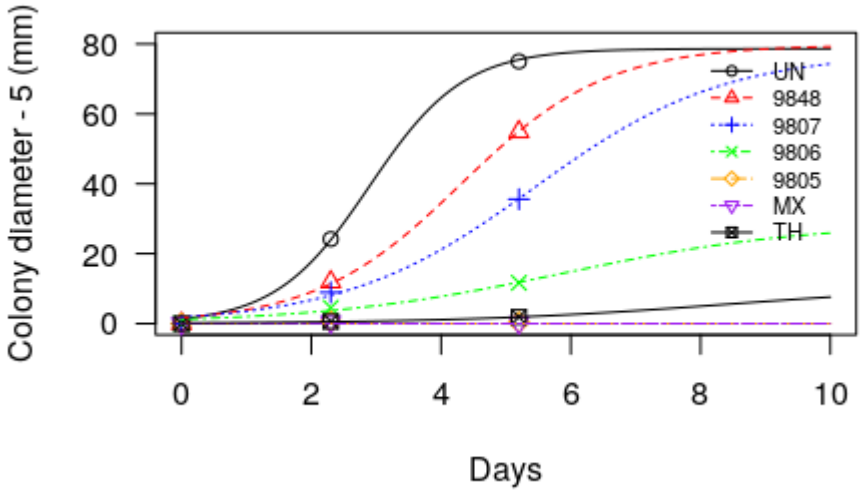

Figure 1. Effect of fungicides on growth of neck rot pathogens (*Botrytis aclada/allii*) on plates of potato dextrose agar (PDA). Points represent the means of six isolates (three *B. aclada* and three *B. allii*) and three product concentrations. Curves represent a three-parameter logistic growth curve. Treatment codes: MX = Maxim 480 FS, UN = Untreated, TH = Thiram, numbered codes are confidential. Note that product 9805 is not visible as it is hidden by MX.

Table 7. Effect of fungicides on mean growth rates of onion neck rot pathogens (*Botrytis aclada/allii*) on pates of potato dextrose agar (PDA). Values represent the means of six isolates (three *B. aclada* and three *B. allii*) and three product concentrations.

Treatment	Growth rate (R_0)	s.e.
Untreated (UN)	1.38	0.35
AHDB9848	0.89	0.05
AHDB9807	0.69	0.05
AHDB9806	0.53	0.1
AHDB9805	0	na
Maxim 480 FS (MX)	0	na
Thiram (TH)	0.49	0.58

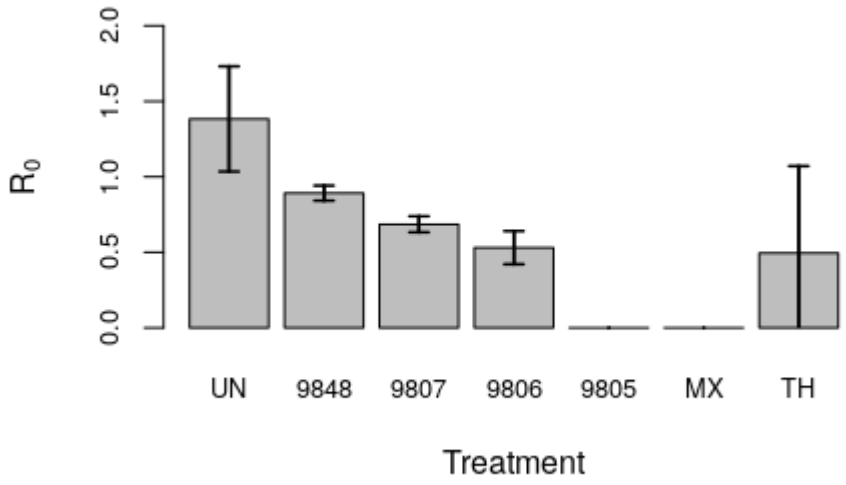

Figure 2. Effect of fungicides on mean growth rates (R_0) of onion neck rot pathogens (*Botrytis aclada/allii*) on pates of potato dextrose agar (PDA). Values represent the means of six isolates (three *B. aclada* and three *B. allii*) and three product concentrations. Error bars represent the standard errors of the means. Treatment codes: MX = Maxim 480 FS, UN = Untreated, TH = Thiram, numbered codes are confidential.

WP3 Seed Tests

Due to limited number of treatment slots, two products in the plate tests were not included: AHDB9848 as it was the least effective, and Thiram (originally included as an old standard) as it is no longer approved. Due to an equipment failure, treatment of one seed lot with AHDB9803 was missing, therefore additional replicates of the other treatment lots were included to compensate.

When recording the seed test plates, it was clear that inoculation of one of the seed lots (S2567) had not been as successful as the others, resulting in much lower and more variable infestation levels (and negative results in the untreated control). Although it did not affect the conclusions, in preliminary data analysis it was clear that inclusion of this seed lot in the data set contributed to poor model fit, and outliers. Therefore for comparability with later results (transmission tests), the results for this lot were excluded from the analysis.

Analysis of deviance indicated significant effects of seed lot (= inoculated *Botrytis* species/strain) and treatment, and that the interaction term was not important. Means (combined across the three seed lots) for each treatment are shown in Table 8 and Fig. 3. All treatments except for hydrogen peroxide (PE) reduced the level of seed infestation compared to the untreated control. The most effective treatments were hot water (HW) (>99% reduction) and the current standard Maxim 480FS (MX) (97% reduction).

The germination data indicated that one treatment (AHDB9803) had a significant adverse effect on germination.

Table 8. Effect of seed treatments on onion seed infestation with neck rot pathogens (*Botrytis aclada/allii*) and germination. Values represent the means of three seed lots (each inoculated with a different *Botrytis* species/strain) and the lower (LCL) and upper (UCL) 95% confidence limits.

Treatment	Infestation (proportion)			Germination (proportion)		
	Mean	LCL	UCL	Mean	LCL	UCL
Hot Water (HW)	0.01	0.00	0.24	0.93	0.86	0.97
Hydrogen peroxide (PE)	1.00	0.97	1.00	0.86	0.78	0.92
Acetic acid (AC)	0.30	0.17	0.47	0.93	0.86	0.97
AHDB9804	0.39	0.24	0.56	0.94	0.87	0.97
Maxim 480 FS (MX)	0.03	0.00	0.18	0.90	0.82	0.95
AHDB9805	0.60	0.46	0.73	0.93	0.87	0.96
AHDB9806	0.80	0.63	0.90	0.95	0.89	0.98
AHDB9807	0.84	0.68	0.93	0.90	0.82	0.95
AHDB9803	0.24	0.12	0.42	0.40	0.30	0.51
Untreated (UN)	1.00	0.97	1.00	0.94	0.89	0.97

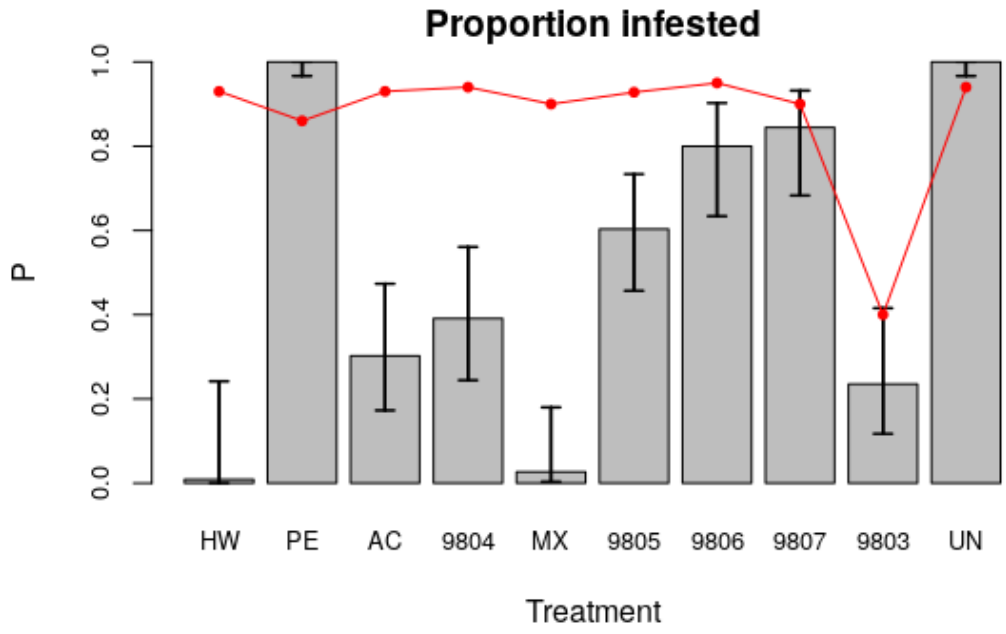

Figure 3. Effect of seed treatments on onion seed infestation with neck rot pathogens (*Botrytis aclada/allii*) and germination. Bars show infestation levels (means of three seed lots, each inoculated with a different *Botrytis* species/strain) with error bars indicating 95% confidence limits. The red points/line show the germination. Treatment codes: HW = Hot water, PE = Hydrogen peroxide, AC = acetic acid, MX = Maxim 480 FS, UN = Untreated, numbered codes are confidential.

WP4 Disease Transmission

Due to the limited treatment slots, two of the seed test treatments were not included to make way for the biological treatments: hydrogen peroxide as it did give any

reduction in seed infestation levels and AHDB9803 as it had an adverse effect on germination.

The seed lot (S2567) identified as having low/variable infestation levels in the seed tests was not used, and was replaced with an additional replicate/block of one the other seed lots (S2568). Thus three seed lots were sown in four blocks: two blocks with S2568, one with S2569 and one with S2570.

Emergence

Analysis of deviance indicated that several treatments had a significant (positive) effect on emergence at 14 d compared to the untreated control (Table 9, Fig 4). Hot water (95%), Maxim 480FS (91%), AHDB9804 (91%), AHDB9805 (92%), AHDB9806 (92%) were significantly better than the untreated control (83%).

During harvesting and post harvest counts, the presence of dead or dying seedlings was occasionally noted in some cells. Often this was associated with visible sporulation of the pathogen on the dying tissues.

There was no evidence of phytotoxicity, i.e. none of the treatments had emergence worse than the untreated control, and there were no differences in appearance or growth rate in the emerged seedlings.

Table 9. Effect of seed treatments on emergence in transmission experiment. Values represent the means of three seed lots (each inoculated with a different *Botrytis* species/strain) and the lower (LCL) and upper (UCL) 95% confidence limits.

Treatment	Emergence (proportion)		
	Mean	LCL	UCL
Hot water	0.95	0.92	0.97
Acetic acid	0.85	0.80	0.89
AHDB9804	0.91	0.87	0.94
Maxim 480FS	0.91	0.87	0.94
AHDB9805	0.92	0.87	0.95
AHDB9806	0.92	0.89	0.94
AHDB9807	0.82	0.77	0.86
AHDB9849[B]	0.84	0.79	0.88
AHDB9855[B]	0.83	0.78	0.87
Untreated	0.83	0.79	0.87

[B] Biological control agent.

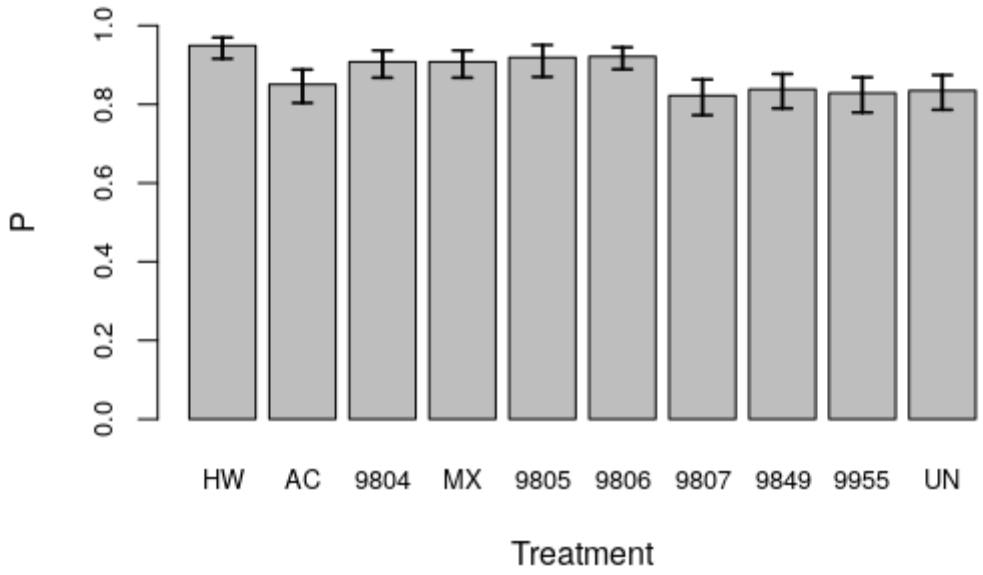

Figure 4. Effect of seed treatments on emergence of onions in transmission experiment. Bars show emergence (means of three seed lots, each inoculated with a different *Botrytis* species/strain) with error bars indicating 95% confidence limits. Treatment codes: HW = Hot water, AC = Acetic acid, MX = Maxim 480 FS, UN = Untreated, numbered codes are confidential.

Transmission

Analysis of deviance indicated that treatments had a highly significant effect on disease transmission from seed to seedling. Differences between seed lots/blocks were not significant. Hot water, Maxim 480FS, Acetic acid, AHDB9804, AHDB9805, AHDB9806 all gave significant reductions in transmission compared to the untreated control. The biggest reduction (>99%) was achieved with hot water treatment (Table 10. Fig. 5)

Table 10. Effect of seed treatments on transmission (proportion of infected seedlings) of onion neck rot pathogens (*Botrytis aclada/allii*) from seed to seedling. Values are the means of three seed lots (each inoculated with a different *Botrytis* species/strain) together with lower (LCL) and upper (UCL) 95% confidence limits

Treatment	Transmission (proportion)			
	Mean	LCL	UCL	% Redn.
Hot water	0.003	0.001	0.012	99.7
Acetic acid	0.085	0.060	0.118	91.5
AHDB9804	0.018	0.010	0.033	98.2
Maxim 480FS	0.159	0.116	0.215	84.1
AHDB9805	0.043	0.029	0.065	95.7
AHDB9806	0.084	0.060	0.117	91.6
AHDB9807	1.000	0.995	1.000	0.0
AHDB9849[B]	0.997	0.995	1.000	0.0
AHDB9855[B]	0.995	0.990	1.000	0.0
Untreated	0.498	0.361	0.654	0.0

[B] Biological control agent.

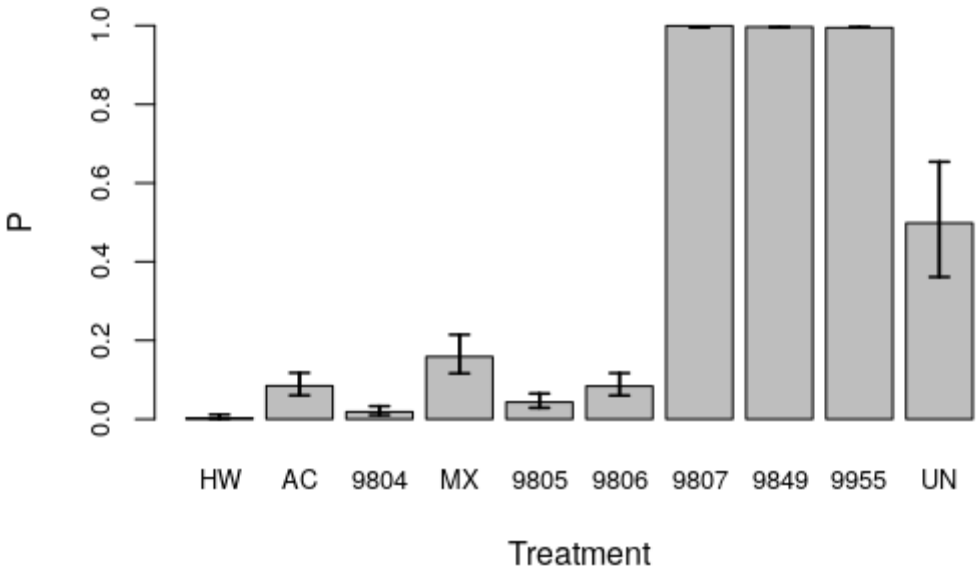

Figure 5. Effect of seed treatments on transmission (proportion of infected seedlings, P) of onion neck rot pathogens (*Botrytis aclada/allii*) from seed to seedling. Values are the means of three seed lots (each inoculated with a different *Botrytis* species/strain), error bars represent the lower and upper 95% confidence limits. Treatment codes: HW = Hot water, AC = Acetic acid, MX = Maxim 480 FS, UN = Untreated, numbered codes are confidential.

Discussion

The results clearly showed that several onion seed treatments can result in significant reductions in both seed infestation with neck rot pathogens and transmission from seed to seedling. Hot water treatment was consistently the most

effective treatment, giving >99% reduction in both seed infestation and transmission from seed to seedling. The current fungicide standard, Maxim 480 FS, three other coded fungicide products AHDB9804, 9805, 9806) and the basic substance acetic acid (vinegar) also gave significant reductions in both infestation and transmission, but were not as effective as hot water. The biological products, which can only be validly tested in transmission experiments did not give any detectable reduction in transmission.

A priori, it would be expected that the ranking of treatments in seed tests and transmission tests would be similar, as transmission from seed-to-seedling is dependent on the proportion of seeds infested and the inoculum dose per seed. Hence the treatments that give the greatest apparent reduction in seed infestation would be expected to also give the greatest reductions in transmission. Whilst this was the case for the hot water treatment, it was not the case for the chemical fungicides. Thus, of the fungicides, Maxim 480 FS was apparently the best when assessed by seed testing, but AHDB9804 was the best when assessed by transmission. Although these differences were relatively small, they may reflect subtle differences in modes of action, plant absorption and translocation, and persistence/degradation amongst the products/formulations.

The plate tests allowed us to screen out one chemical product with little activity against the target pathogens. Likewise the direct seed tests allowed us to screen out a further two products.

Ultimately management of a seed borne disease is achieved by reducing seed to seedling transmission to the lowest possible levels. Thus while the plate tests for fungicides and the seed tests can be used to screen out the most ineffective products/treatments, they should not be considered as definitive indicators of performance as seed treatments.

These results show that high levels of *Ba* on seed may result in reduced emergence, thus the most effective treatments not only reduced transmission of *Ba* but also improved germination and emergence compared to the untreated control.

One coded treatment (AHDB 9803) had a detrimental effect on germination, this was a proprietary physical treatment that had not been specifically optimised for onions. It is possible that improvement can be achieved following optimisation.

Two basic substances, hydrogen peroxide (PE) and acetic acid (AC) were included in the treatments. In both cases there was no optimisation of the treatment conditions, and a conservatively short treatment time of 10 minutes was used in both cases. Thus whilst hydrogen peroxide did not give any apparent reduction in levels of *Ba*, it is possible that with a longer treatment time, some improvement in efficacy can be achieved. Similarly, acetic acid appears to be a promising treatment and efficacy could potentially be improved further with a longer treatment time.

Conclusions

- The most effective seed treatment for neck rot control was hot water (>99% reduction in transmission).
- Significant reductions in disease transmission were achieved with three coded chemical fungicides; they were comparable to the current standard onion seed treatment (Maxim 480 FS), but were not as good as hot water.
- The basic substance vinegar was as effective as the current standard treatment and chemical fungicides.

- Ranking of treatments was not the same in seed tests and transmission tests, i.e. some products performed relatively better in the transmission tests than in direct seed tests.
- Definitive determination of seed treatment efficacy should be based on evaluation of seed-to-seedling transmission rather than seed tests.

Acknowledgements

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Appendix

a. Trial/Crop diaries

Exp:	1260	Plate tests
Date	Days	Activity
16/11/20	-2	Media prepared
18/11/20	0	Plates inoc with 5 mm plug of culture, ~11.00
20/11/20	2.3	Plates recorded 17:00 on
23/11/20	5.2	Plates recorded 16:00 on
03/12/20	15	Plates discarded

Exp:	1264	Seed tests
Date	Days	Activity
12/11/20	-29	Seed inoculation completed
03/12/20	-8	HW treatment applied
04/12/20	-7	Other treatments applied
09/12/20	-2	Media prepared
11/12/20	0	AHDB903 treated seed rec'd
11/12/20	0	Seed plated on KRZ medium. Positive control samples also plated
18/12/20	7	First recording
24/12/20	13	Second recording 15:00 on.
24/12/20	13	Exp completed. Plates discarded

Exp:	1265	Transmission experiment
Date	Days	Activity
12/11/20	-67	Seed inoculation completed
03/12/20	-46	HW treatment applied
04/12/20	-45	Other treatments applied
11/12/20	-38	AHDB903 treated seed rec'd
11/12/20	-38	Seed tested (except for biological treatments)
11/12/20	-38	Seed stored in fridge
10/01/21	-8	Irrigation system set up
15/01/21	-3	Bottles of seed weighed

18/01/21	0	Seed sown GH E8
18/01/21	0	Trays set out according to randomisation plan, and initial watering ~5min
19/01/21	1	Watering 1 min 08:00, 1 min 15:00 auto
19/01/21	1	Temp adjusted 15/18 night/day
22/01/21	4	Bottles of seed reweighed post sowing
22/01/21	4	All look ok.15:00 Blockage in spray head 4 rh side. Cleared.
22/01/21	4	All trays rotated clockwise 1 position
26/01/21	8	Starting to emerge.
26/01/21	8	All trays rotated clockwise 1 pos. Watering still auto 1 min am, 1 min pm
29/01/21	11	Watering looks even. Trays rotated 1 unit clockwise.
02/02/21	15	Most approaching crook stage with cotyledon still sharply bent. Emergence counts on first 15 cells in each tray. Trays rotated 1 unit
02/02/21	15	Seem very wet, too wet? Omit pm watering from now on, and judge need for tomorrow.
08/02/21	21	1st TL just visible. Cotyledons straight. All trays rotated 1 unit
08/02/21	21	Some cells with dead/dying seedlings
11/02/21	24	Quite a few trays drying out, so will water at 15:00
12/02/21	25	Checks, rotated trays, some beginning to dry out so extra water 1 min
16/02/21	29	Harvested B4 and B3 → stored in dark at RT
17/02/21	30	Harvested B2 and B1 → stored in dark at RT
19/02/21	32	Final recording of emergence/stubs, residue disposed into black bags
23/02/21	36	GH disinfected with Jet 5
24/02/21	37	Irrigation kit dismantled
24/02/21	37	Recorded first harvest B4 S2568, and B3 S2569
26/02/21	39	Recorded second harvest B2 S2568 and B1 S2570

b. Photographs

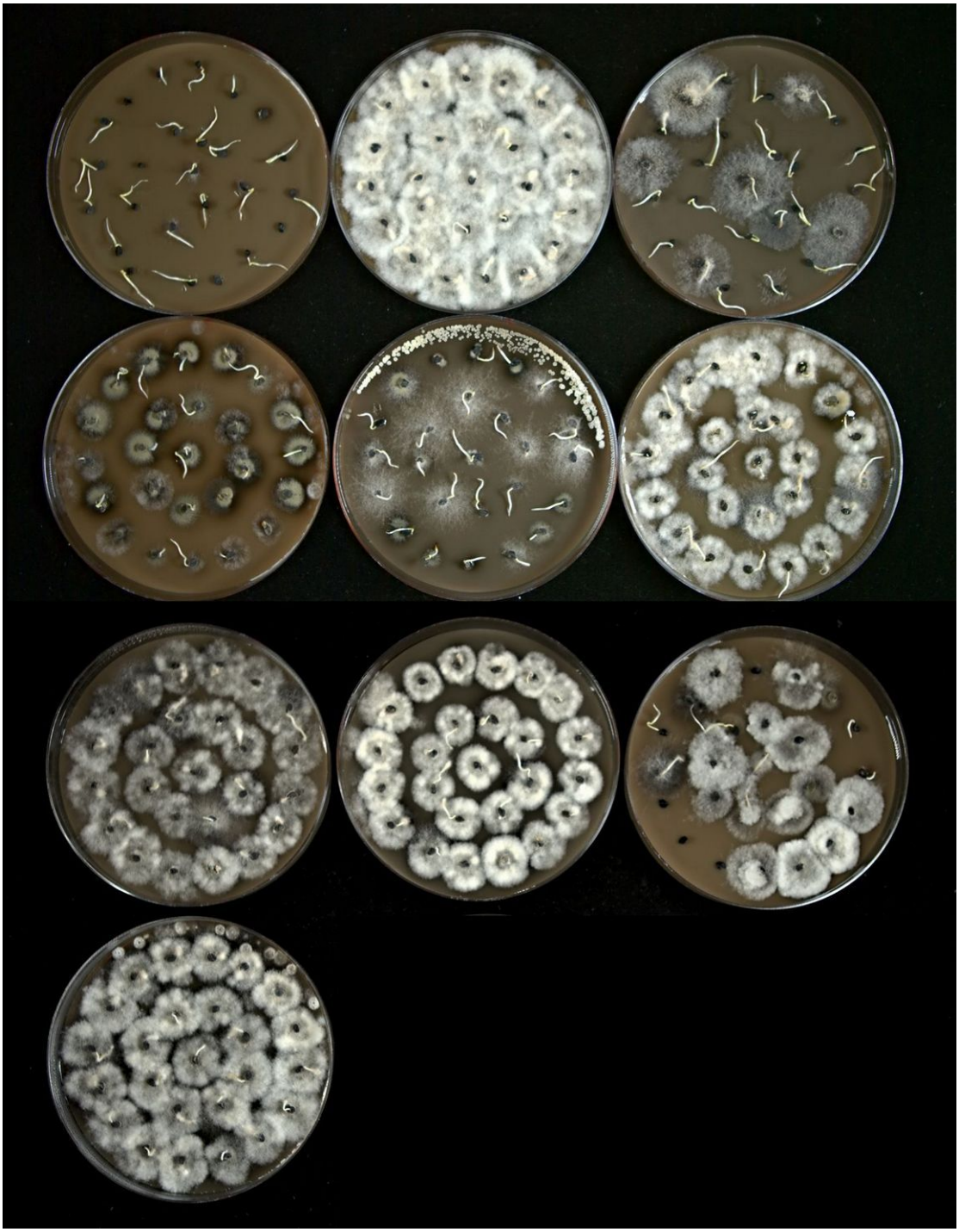

Image 1. Example seed test plates. From TL to BR Treatments 1, 2, 3, 4, 5, 6, 7, 8, 9, 12 (see Table 3 for treatment numbers). Treatment 12 (BL) is the untreated control.

Image 2. Overview of experimental layout for transmission experiment.

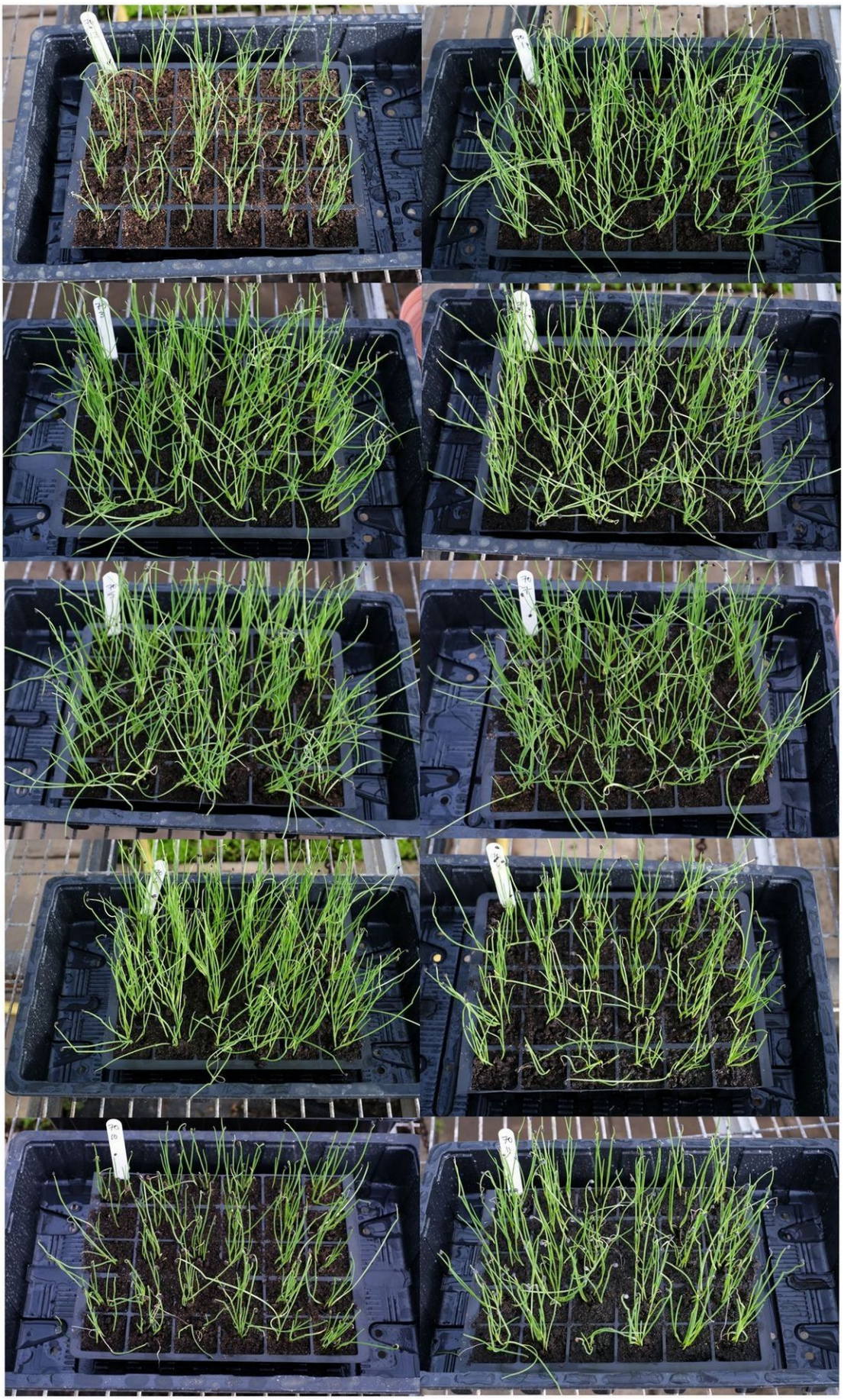

Image 3. Example plots from one block just before harvest. TL to BR: Treatment numbers 12, 1, 3, 4, 5, 6, 7, 8, 10, 11. See Table 3 for treatment numbers. Treatment 12 (TL) is the untreated control.

c. Raw data

Exp	1264	Seed tests	
Lot	Tcode	Ba	N seeds
2567	UN	0	50
2567	9803	23	100
2567	9804	0	74
2567	9805	0	75
2567	9806	0	75
2567	9807	0	75
2567	AC	1	75
2567	HW	0	75
2567	MX	0	75
2567	PE	29	75
2568	UN	50	50
2568	9804	35	75
2568	9805	75	75
2568	9806	75	75
2568	9807	75	75
2568	AC	31	75
2568	HW	1	75
2568	MX	1	75
2568	PE	75	75
2569	UN	50	50
2569	9803	47	100
2569	9804	27	75
2569	9805	31	150
2569	9806	30	75
2569	9807	40	75
2569	AC	26	75
2569	HW	0	75
2569	MX	1	75
2569	PE	75	75
2570	UN	50	50
2570	9803	0	100
2570	9804	26	75
2570	9805	75	75
2570	9806	75	75
2570	9807	75	75
2570	AC	11	75

2570	HW	1	75
2570	MX	4	75
2570	PE	75	75

Exp:	1265	Transmission	
Block	Lot	Tcode	p
1	2570	UN	0.556
1	2570	9804	0.000
1	2570	9805	0.043
1	2570	9806	0.148
1	2570	9807	1.000
1	2570	9849	0.998
1	2570	9955	0.997
1	2570	AC	0.089
1	2570	HW	0.000
1	2570	MX	0.263
2	2568	UN	0.511
2	2568	9804	0.028
2	2568	9805	0.059
2	2568	9806	0.069
2	2568	9807	0.987
2	2568	9849	0.996
2	2568	9955	0.958
2	2568	AC	0.091
2	2568	HW	0.006
2	2568	MX	0.249
3	2569	UN	0.437
3	2569	9804	0.021
3	2569	9805	0.020
3	2569	9806	0.072
3	2569	9807	0.987
3	2569	9849	0.957
3	2569	9955	0.978
3	2569	AC	0.070
3	2569	HW	0.000
3	2569	MX	0.061
4	2568	UN	0.511
4	2568	9804	0.028
4	2568	9805	0.059

4	2568	9806	0.069
4	2568	9807	0.987
4	2568	9849	0.996
4	2568	9955	0.958
4	2568	AC	0.091
4	2568	HW	0.006
4	2568	MX	0.249

d. Trial design

Exp:	1265	Transmission		
Block	Plot	Treat	Species	Seed lot
1	1	6	Bac	S2568
1	2	4	Bac	S2568
1	3	11	Bac	S2568
1	4	7	Bac	S2568
1	5	3	Bac	S2568
1	6	12	Bac	S2568
1	7	10	Bac	S2568
1	8	8	Bac	S2568
1	9	5	Bac	S2568
1	10	1	Bac	S2568
2	11	6	Bal	S2569
2	12	8	Bal	S2569
2	13	11	Bal	S2569
2	14	7	Bal	S2569
2	15	5	Bal	S2569
2	16	1	Bal	S2569
2	17	10	Bal	S2569
2	18	3	Bal	S2569
2	19	4	Bal	S2569
2	20	12	Bal	S2569
3	21	7	Bac	S2568
3	22	10	Bac	S2568
3	23	12	Bac	S2568
3	24	6	Bac	S2568
3	25	4	Bac	S2568
3	26	1	Bac	S2568
3	27	8	Bac	S2568
3	28	3	Bac	S2568
3	29	11	Bac	S2568
3	30	5	Bac	S2568
4	31	8	Bal	S2570

4	32	12	Bal	S2570
4	33	4	Bal	S2570
4	34	11	Bal	S2570
4	35	5	Bal	S2570
4	36	1	Bal	S2570
4	37	6	Bal	S2570
4	38	3	Bal	S2570
4	39	7	Bal	S2570
4	40	10	Bal	S2570

e. ORETO certificate should be pasted in at end.

Certificate of

Official Recognition of Efficacy Testing Facilities or Organisations in the United Kingdom

This certifies that

Warwick Crop Centre, School of Life Sciences

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
Biologicals and Semiochemicals

Date of issue: **6 October 2017**
Effective date: **20 March 2017**
Expiry date: **19 March 2022**

Signature Alison Richardson
Authorised signatory

Certification Number
ORETO 381

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Chemicals Regulation Division

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