Project title:	AHDB Spinach damping-off seed treatment trials
Project number:	FV 464b
Project leader:	Dave Kaye, ADAS RSK Ltd., Horticulture
Report:	Final Report November 2021
Previous report:	N/A
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Location of project:	Various growers
Industry Representative:	Liz Johnson, L J Technical Consultancy Ltd.
Date project commenced:	01/06/2021

DISCLAIMER

The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Moreover, it should be noted that because of the unreplicated nature of the field trials in this project, observations and discussion of possible treatment effects relate to trends in the data rather than outputs from statistical analysis. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

This project incorporated grower-led field trials. Products evaluated for their effects on crop performance included biostimulants as well as plant protection products (authorised and in *development). No endorsement or recommendation of named products is intended nor is any criticism implied of alternative, untested products.*

The products listed in this report are not necessarily authorised as plant protection products in UK and mention of a product does not constitute a recommendation for its use against specific plant pathogens. Plant protection products must only be used in accordance with the authorised conditions of use.

Any product marketed for use specifically against Pythium species or any other plant pest/disease would require an authorisation under the Plant Protection Products Regulations/Regulation (EC) 1107/2009 before they are placed on the market for this use.

Regular changes occur in the authorisation status of biocides and plant protection products. For the most up to date information, please check with your professional supplier, BASIS registered adviser or the Chemical Regulation Division (CRD) of HSE (https://www.hse.gov.uk/crd/).

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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GROWER SUMMARY

Headline

Grower-led field trials provided a useful comparison of the effects of available and future seed treatments on spinach crop performance. The results (not statistically analysed) could help to guide growers and agronomists when considering management options for spinach damping-off.

Background

Increasing issues of damping-off diseases have been identified by the leafy salads industry since the loss of thiram as a seed treatment and metalaxyl-M as a seed treatment for outdoor drilled crops. Pathogens included in the damping-off complex for spinach are known to include *Pythium*, *Fusarium* and *Rhizoctonia* species and can devastate crops. There was a high incidence of damping-off in spinach fields in 2020. This grower-led demonstration trial was requested by spinach growers to investigate the use of a range of seed treatments to minimise crop losses. Conventional and biological plant protection products in the pipeline for approval as well as commercially available products and biostimulants were tested alongside each other for their effect on crop performance in the field. The trial considered the suitability and efficacy of the seed treatments for use in the near future.

The aim of the demonstration trials was to compare the different seed treatments for effects on spinach emergence, crop establishment, quality, yield and damping-off.

Summary

Methods

Two sets of trials were conducted:

1) Field demonstration plot trials – on grower's farms, coordinated by RSK ADAS Ltd.
2) Bioassays – conducted by Tim Pettitt at Eden Project Learning.

1) Field demonstration plot trials in 2021

Site locations

- Ten sites were provided by nine growers across England.
- Demonstration plots were placed in areas with a known history of damping-off or in conditions conducive to disease development i.e. wet or shady.

Seed treatments

- Conducted by Elsoms Seeds Ltd. using film coating process.
- Seeds delivered directly to growers when treated.

Table 1. Treatments used in trial with AHDB codes where required.

No.	Treatment	Active ingredient	Type
1	Untreated	-	Control
2	Maxim 480FS	Fludioxonil	Conventional fungicide (seed treatment)
3	Integral Pro	Bacillus amyloquefaciens	Biological fungicide (bacterial seed treatment)
4	AHDB 9763		Biostimulant: bacterial species (liquid microbial fertilizer)
5	AHDB 9733		Biostimulant: mycorrhizal, bacterial and fungal species (seed treatment)
6	AHDB 9848		Conventional fungicide (seed treatment)
7	AHDB 9732		Biostimulant: Mineral + biostimulant (biostimulant seed treatment)
8	AHDB 9734		Biological fungicide (bacterial seed treatment)
9	Priming		Physical

Germination test on seed conducted by Elsoms Seeds Ltd.

- 100 seeds, incubated in moist chamber for 28 days and assessed weekly.
- Scored as germinated/ungerminated (counts)

Trial design

- One row per treatment (nine rows total).
- Treatments positioned in random order (single replicate/site sowing).
- Growers asked to sow up to three successive trials.
- 16 trials across all sites and growers were conducted.

Field assessments

- Assessments timed to coincide with approximately 50% emergence, 100 % emergence, 10-15 days after full emergence (100%+10-15 days) and at harvest.
- Assessments conducted:

1. % Healthy plants – Quadrat counts x3
2. % Damping-off – Quadrat counts x 3
3. Cover - %, whole plot
4. Vigour – whole plot, 0-10 scale (5 was average vigour)
5. Phytotoxicity – whole plot, 0-10 scale. 0 = 100% crop kill, 10 = no damage (Table 4)
6. Yield at harvest, if possible.
7. Comments – grower comments on treatment performance

Crop destruction

- Treatment with AHDB 9848 and AHDB 9734 required the crop to be destroyed.

Data analysis

- Percentage health and damped-off calculated to account for variable quadrat sizes.
- Results grouped by closest emergence category (50%/100%/100%+10-15 days/harvest).
- Grower comments given traffic light colour based on whether negative (red)/intermediate (amber)/positive (green).

2) Bioassay

Isolate

- A field site soil sample was used for isolation of potential pathogens.
- Isolates were screened for pathogenicity on spinach seedling leaves.
- One pathogenic isolate was tentatively identified as *Pythium ultimum* based on morphology.
- Bulk inoculum was prepared in oatmeal/sand mixture to a concentration of 1.6×10^3 CFU $^{g-1}$ for the first bioassay trial and 2.2×10^3 CFU $^{g-1}$ for the second

Seed sowing

- Oatmeal/sand inoculum was placed into sterilised mushroom punnets (250 g inoculum per punnet).

- There were five seeds per cell, sown at 10 mm depth in Levington Advance F1 seed and modular growing medium into cell plug trays with 3x4 cells.
- Three replicate inoculated and 3 replicate uninoculated control trays/punnets were set up for each treatment.
- Trays were arranged in 3 randomised blocks.

Assessments

- Number of seeds germinated (count).
- Number of seeds surviving to emergence of first true leaves (count).

Results

1) Field demonstration plot trials

Germination test *in vitro*

- Most treatments had comparable germination rates to the untreated control (range 95-97%).
- Germination rates for AHDB 9848 (conventional fungicide) and AHDB 9763 (biostimulant) were slightly lower at 93% and 94%, respectively.

Percentage damping-off (Figure 1)

- Damping-off incidence was generally very low (<10% plants affected) across all trial sites, and the causal pathogens were not determined.
- Lowest damping-off incidence was observed for AHDB 9848 (conventional fungicide), AHDB 9763 (biostimulant) and seed that was primed.

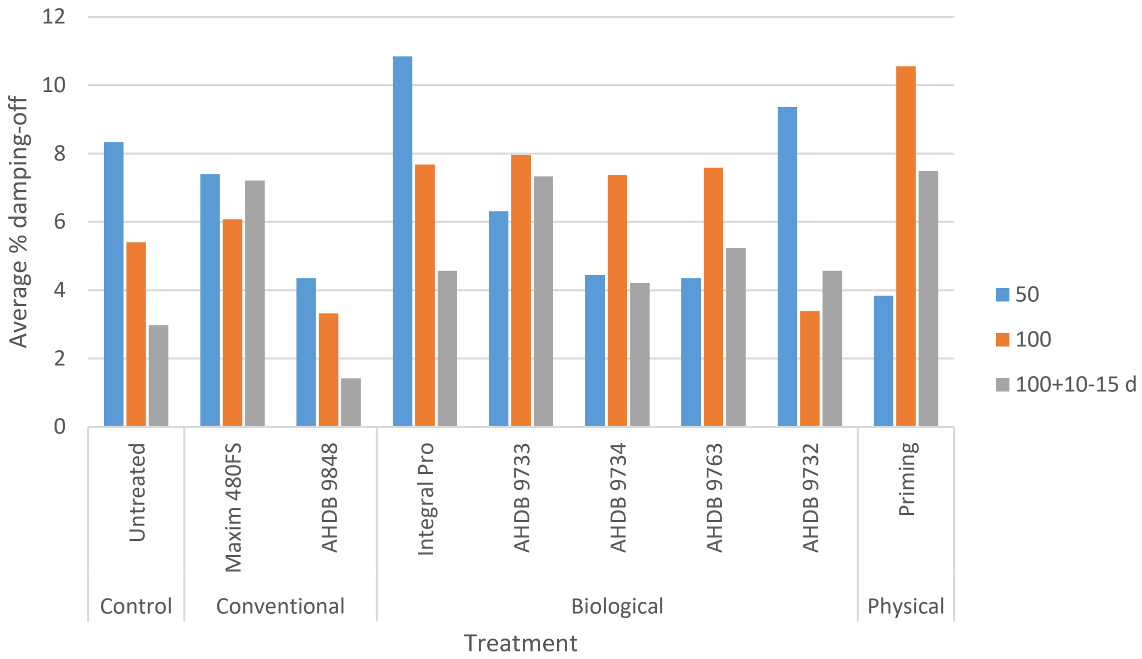

Figure 1. Effect of seed treatment on average percentage damping-off when assessed at different percentage emergence and development timepoints (50% emergence, 100% emergence, 100% emergence + 10-15days + Harvest).

Vigour

Under low damping-off risk conditions of 2021:

- Vigour scores did not vary widely between treatments, ranging between scores of 4.3 to 6.9.
- Highest vigour scores were obtained from plots with seed treated with AHDB 9732 biostimulant), while vigour in Maxim-treated plots was consistently the lowest.
- AHDB 9848 (conventional fungicide), AHDB 9763 (biostimulant) and AHDB 9734 (biofungicide) were as good as the untreated control.

Percentage ground cover (Table 2)

The conventional treatment AHDB 9848 gave slightly higher ground cover than the untreated control.

- Out of the biostimulants, the highest % ground cover was obtained with AHDB 9732.
- Biofungicide AHDB 9734 was the better performer of the two biofungicides, but below that of the untreated.

- Primed seed plots had marginally better cover than the untreated control by harvest.

Table 2. Effect of treatment on average cover, scored as percentage of whole plot at emergence assessment timepoints across all trials.

	Treatment	**% ground cover at different assessment time points (% emergence)**
		Harvest
Control	Untreated	91.4
Conventional fungicides	Maxim 480FS	60.8
	AHDB 9848	95
Biological fungicides	Integral Pro	67.5
	AHDB 9734	79.2
Biostimulants	AHDB 9732	83.3
	AHDB 9733	65
	AHDB 9763	75
Physical	Priming	93.3

Phytotoxicity

- Classic scorching symptoms were not observed from any treatments but there was some impact on growth. This metric was open to interpretation by grower-assessors so should be treated with caution.
- AHDB 9848 plants had the best appearance over the assessment period to harvest.
- Maxim 480 FS had the poorest appearance throughout.

Yield

- Data from only one grower trial was considered as the others were too sparse to be harvested
- AHDB 9732 (biostimulant), AHDB 9848 (conventional fungicide) and AHDB 9763 (biostimulant) had the best yields.

Grower comments on treatment performance (Table 3)

- Conventional fungicide AHDB 9848, biostimulant AHDB 9732 and priming were all reported to perform well, while two products were clearly poor (Table 2).

Table 3. Comments from growers. For individual comments see Appendix A.

Product type	Treatment	Comment summary
Control	Untreated	Mostly positive
Conventional	Maxim 480 FS	All poor
	AHDB 9848	Mostly positive
Biological fungicide	Integral Pro	Mostly poor, some positive
	AHDB 9733	All poor
	AHDB 9734	Mostly poor, some positive
	AHDB 9763	Mostly poor, some positive
Biostimulant	AHDB 9732	Mostly positive
Physical	Priming	Mostly positive

2) Bioassay trials

- Very little damping-off was recorded in the first trial, which had low inoculum levels. . The second trial with a higher inoculum level had higher disease incidences.
- At higher inoculum levels, priming and AHDB 9848 gave better emergence than the other treatments and the untreated control.
- At higher inoculum levels, AHDB 9848 and AHDB 9733 had the best seedling survival, albeit low (Figure 2).

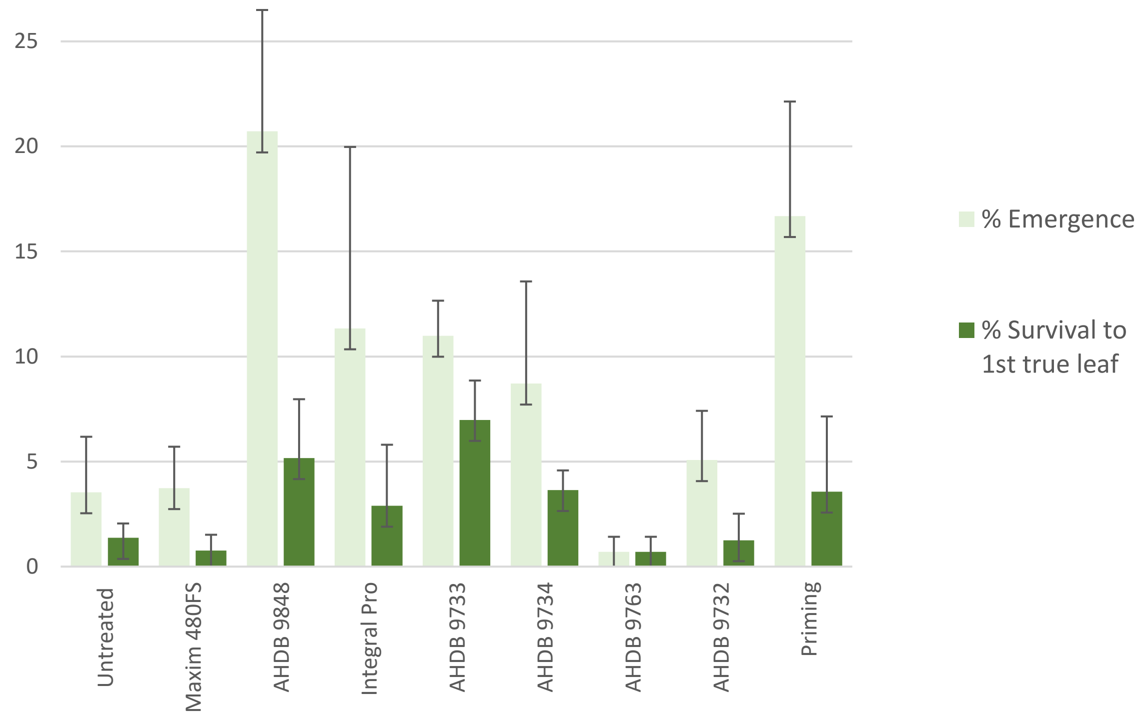

Figure 2. Experiment 2 comparisons between seed treatments of % emergence and of germination (survival to emergence of first true leaves) of inoculated treatments as a percentage of uninoculated controls (bars are standard errors of the means of three counts).

Conclusions

In terms of overall crop performance, AHDB 9848 was the better conventional fungicide treatment in both field trial and bioassay.

AHDB 9732 was the best of the biostimulants and the increase in vigour it seemed to induce may have helped seedling survival.

Grower comments aligned well with the field data collected.

A replicated trial would be valuable to determine if the differences between treatments are statistically significant and real effects seen.

SCIENCE SECTION

Introduction

Increasing issues of damping-off diseases have been identified by the leafy salads industry since the loss of thiram as a seed treatment, and loss of authorisation for the use of Apron XL (metalaxyl-M) seed on outdoor drilled crops. Pathogens included in the damping-off complex for spinach are known to include *Pythium*, *Fusarium* and *Rhizoctonia* species and can devastate crops. There was a high incidence of damping-off in spinach fields in 2020.

This grower led demonstration trial was requested by spinach growers to investigate the use of a range of seed treatments to minimise crop losses. Conventional and biological plant protection products and growth stimulators (biostimulants) were tested alongside each other for their effect on spinach production in the field. Treatments included commercially available products and as yet unapproved products. The trial objective was to consider the suitability and efficacy of the seed treatments for use in the near future.

This work was complemented by bioassay trials conducted by Tim Pettitt, Eden Project Learning. The materials and methods and results sections are divided into two sections: 1) Field demonstration plot trials, 2) Bioassays.

Materials and methods

1) **Field demonstration plot trials**

Trial location

Nine growers distributed across England agreed to participate in these demonstration trials, with one grower providing 2 sites, giving a total of 10 sites.

Growers were asked to place demonstration plots in areas with a known history of damping-off disease and where possible in an area where variation across the trial is kept to a minimum. Spinach plants grown under wet or shady patches are at greater risk of developing root rot diseases as these conditions favour pathogens such as *Pythium*.

Seed treatments

A mixture of chemical, biological and biostimulant treatments were selected for evaluation in this work (Table 4).

Table 4. Treatments trialled in this study. Seed treatments were conducted by Elsoms Seeds Ltd.

No.	Treatment	Type
1	Untreated	Control
2	Maxim 480FS	Conventional fungicide (seed treatment)
3	Integral Pro	Biological fungicide (bacterial seed treatment)
4	AHDB 9763	Biostimulant: bacterial species (liquid microbial fertilizer)
5	AHDB 9733	Biostimulant: mycorrhizal, bacterial & fungal species (seed treatment)
6	AHDB 9848	Conventional fungicide (seed treatment)
7	AHDB 9732	Biostimulant: Mineral + biostimulant (biostimulant seed treatment)
8	AHDB 9734	Biological fungicide (bacterial seed treatment)
9	Priming	Physical

Seed treatment was conducted by Elsoms Seeds Ltd. using a film-coating process and treated seed was delivered directly to the host growers when treatments completed. There was a slight delay in delivery after treatment as treatment 9, priming, takes up to 6 weeks to complete.

Germination test on treated seed

Immediately following treatment, *in vitro* germination tests were conducted by Elsoms Seeds Ltd according to their in-house protocols. Briefly, 100 seeds were germinated in chambers lined with moist filter paper and incubated for up to 28 days. Germinated and ungerminated seeds were counted after 7, 14, 21 and 28 days.

Trial design

Each demonstration trial consisted of 9 full rows, one row per treatment (no replication). Treatments were allocated at random to rows. For each subsequent sowing a new randomised order was used. Growers were asked to sow up to three successive trials to maximise the chances of the trial coinciding with conditions conducive to damping-off. A total of 16 trials in total were conducted by the 9 growers over the 10 sites.

Sowing 1	1 Untreated	9 Priming	5 AHDB 9733	4 AHDB 9763	6 AHDB 9848	7 AHDB 9732	3 Integral Pro	8 AHDB 9734	2 Maxim 480FS

Sowing 2	7 AHDB 9732	6 AHDB 9848	1 Untreated	3 Integral Pro	9 Priming	2 Maxim 480FS	8 AHDB 9734	4 AHDB 9763	5 AHDB 9733

Sowing 3	2 Maxim 480FS	4 AHDB 9763	8 AHDB 9734	7 AHDB 9732	5 AHDB 9733	3 Integral Pro	9 Priming	1 Untreated	6 AHDB 9848

Figure 3. Randomisation used for planting of treatments at each sowing date

Montego, a summer drilling variety of spinach, known to be susceptible to damping-off was selected to be used by all growers in this trial. A single batch was treated and distributed to all growers to use in trials to ensure uniformity.

Field assessments

Regular assessments of trials were performed timed to coincide with approximately 50% emergence, 100% emergence, 100%+10-15days and Harvest across the whole trial. Not all time points were assessed by all growers in each trial.

1. Healthy plants – Quadrat counts x3
2. Damping-off – Quadrat counts x 3
3. Ground cover - %, whole plot
4. Vigour – whole plot, 0-10 scale (5 is average vigour)
5. Phytotoxicity – whole plot, 0-10* scale (Table 5)

*8 is considered to be an acceptable level of damage and marketable.

Growers were also asked to record any symptoms of phytotoxicity such as stunting, leaf twisting/distortion, yellowing etc.

6. Yield – this was not a requirement of the trial but desirable if data available.
7. Comments – growers were asked to comment on the performance of each treatment in the trial.

Table 5. Phytotoxicity scale used in field assessments

Crop tolerance score	Equivalent to crop damage (% phytotoxicity)
	complete crop kill 100%
1	80-95% damage
2	70-80%
3	60-70%
4	50-60%
5	40-50%
6	25-40%
7	15-25%
8*	10-15%
9	5-10%
10	no damage

Crop destruction

Spinach produced from seed treated with most of the test products did not require crop destruction and could be marketed if sufficient yield. However, plots treated with AHDB 9848 and AHDB 9734 were required to be destroyed.

Data analysis

There was some variability in when observations were recorded in terms of emergence. Results were 'grouped into the nearest emergence category (50%/100%/100+10-15/harvest) for comparisons. As different size quadrats were used for assessments by different growers the percentage healthy and damped off seedlings was calculated for each trial. These were averaged across trials for each emergence timepoint for comparison. As this was an unreplicated trial it was not possible to do statistical analysis on the data. Grower comments on treatments were scored with a traffic light system based on whether the comment was negative or positive or neutral - red: negative comment, amber: intermediate, green: positive comment. The comments for each treatment were compared in a qualitative way to see the overall assessments across all growers and then categorised as red: mostly poor, amber: mostly poor, some positive, green: mostly positive.

2) Bioassays

Pythium damping-off isolates were taken from a sample of soil plus diseased crop debris sent late season from one of the Midlands field trial sites where severe damping-off disease was seen. Isolates were taken by directly plating pieces of crop debris onto oomycete selective agar and by 'baiting' aliquots of the soil sample using transplanted 2-week-old spinach seedlings which were then plated onto oomycete selective agar after one week.

Pythium isolates were screened for pathogenicity by detached leaf assay procedure of Pettitt *et al*. (2011) using first true spinach seedling leaves. 'Pathogenic' isolates were re-isolated from the leading edge of necrosis and grown on V8 agar and autoclaved grass leaves in sterile distilled water to generate structures for identification. All pathogenic isolates were tentatively identified as *Pythium ultimum* based on morphology. One cleaned isolate was selected and grown on a 10% oatmeal/90% sand mix to prepare bulk inoculum for seedling infection trials. After 7 days growth at room temperature, the oatmeal sand medium contained plentiful active *Pythium* mycelium and was ready for dilution to final concentration with sterile sand and placement in sterilised (3% sodium hypochlorite 10 minutes followed by thorough rinsing with potable mains water) mushroom punnets at a rate of 250g punnet^{-1} (Figure 4a). The inoculum concentration of the soil sample mentioned above was determined by dilution plating (Hunter *et al*., 2006) and found to be 3.31 x 10^3 CFU g^{-1} dry weight of soil. As this was a field with a very high disease incidence, sterile sand was added to the inoculum mix to give an inoculum concentration of approximately 1.6 x 10^3 CFU g^{-1} for the first trial (bioassay) which was then increased to approximately 2.2 x 10^3 CFU g^{-1} for a second trial.

Seeds were sown 5 per module at depth of 10 mm in Levington Advance F1 seed and modular growing medium, contained in TEKU BP series 104 cell plug trays trimmed to 3 x 4 cells to sit on the sand layer inside a punnet (Figure 4b). In each experiment each treatment was set up in three replicate inoculated and 3 replicate uninoculated control trays/punnets and these were arranged in three randomised blocks.

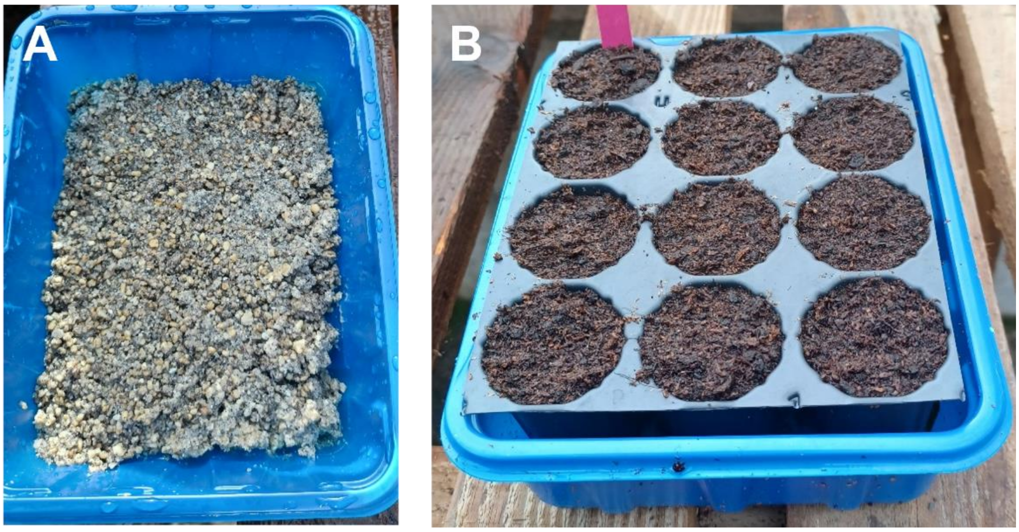

Figure 4. *a) Sterilised mushroom punnet containing 250g dose of* Pythium *inoculum in sand/oatmeal medium. b) Filled and sown 3 x 4 cell plug tray segment placed on oatmeal layer within mushroom punnet illustrated in 1a.*

Table 6. Seed treatment codes used for bioassay trials.

Treatment No.	Treatment code	Treatment type
1	Untreated	Control
2	Primed	Physical
3	AHDB 9763	Biological
4	AHDB 9734	Biological
5	AHDB 9848	Conventional
6	AHDB 9732	Biological
7	Maxim 480 FS	Conventional
8	Integral Pro	Biological
9	AHDB 9733	Biological

Results

1) Field demonstration plot trials

It should be noted that because of the unreplicated nature of the field trials in this project, observations and discussion of possible treatment effects relate to trends in the data rather than outputs from statistical analysis. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

Germination test

Initial vigour at 7 days was increased for most treatments when compared to the untreated control, with the exception of priming which was at an equivalent level to the control. However, in the 2nd, 3rd and final assessments there was a slight negative impact of the treatments on the number of seeds germinated when compared with the untreated control (Figure 5)

AHDB 9848 and AHDB 9763 had a negative impact on final germination percentage. Other treatments were slightly lower than the untreated control, but still maintained relatively high germination rates of 95% or above (Table 7).

Table 7. Percent germination of seeds after treatment. Tests conducted *in vitro* by Elsoms Seeds Ltd.

				% Germination			
Type	**Treatment**	**Rate**	**Lot number**	**7 day count**	**2nd count**	**3rd count**	**Final count**
Control	Untreated	n/a	E72622	89	97	97	97
Conventional fungicide	Maxim 480FS	0.1L per 100kg seed	E72623	90	95	95	95
	AHDB 9848	10 ml per Kg seed	E72627	90	92	93	93
Biological fungicide	Integral Pro	0.4ml per 200g seed	E72624	93	95	96	96
	AHDB 9734	_	E72629	92	95	96	96
Biostimulant	AHDB 9732	1.5L per 100 Kg seed	E72628	92	96	96	96
	AHDB 9733	4ml per Kg seed	E72626	92	96	96	96
	AHDB 9763	5g per Kg seed	E72625	91	92	93	94
Physical	Priming	n/a	E72857	89	95	96	96

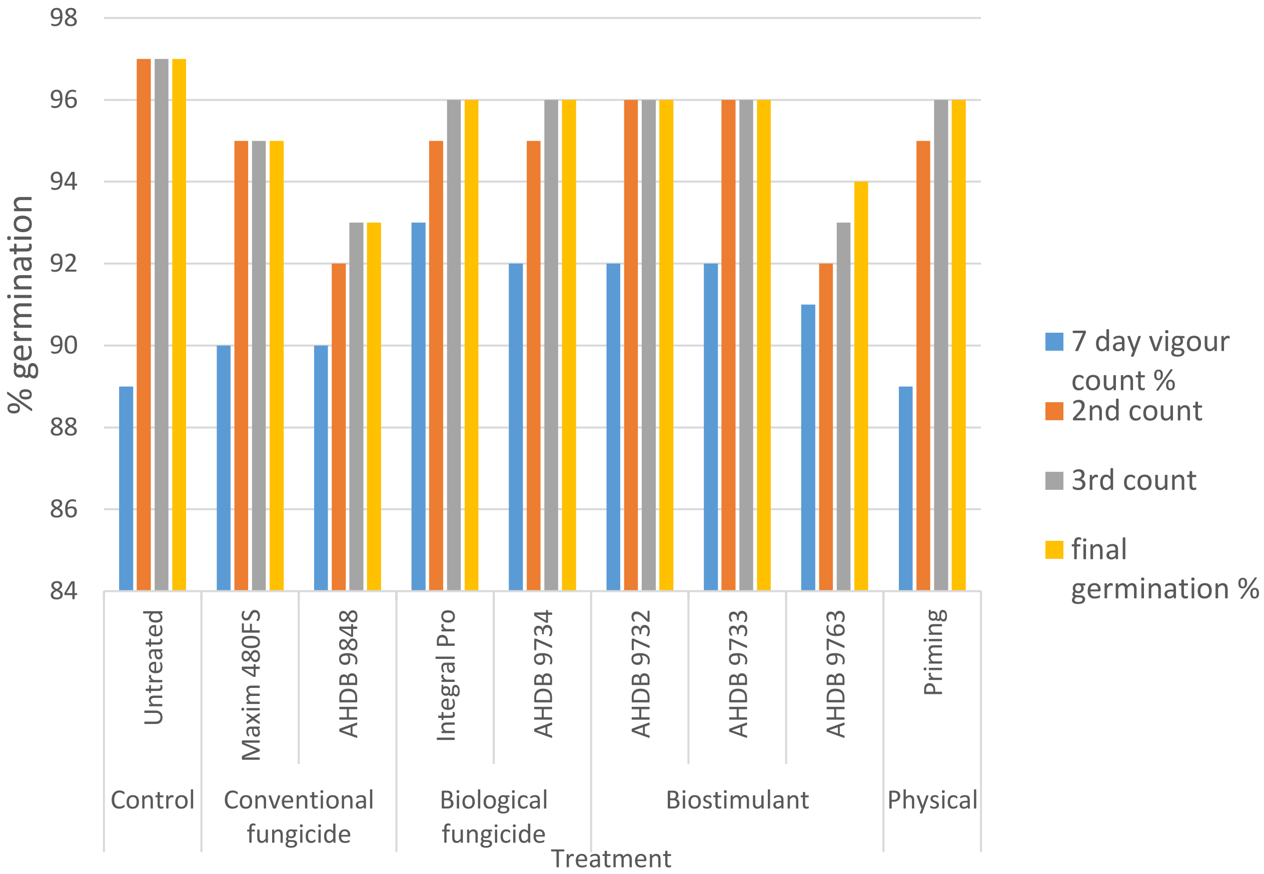

Figure 5. Effect of seed treatment on *in vitro* germination immediately after seed treatment. Seeds were assessed every 7 days for 28 days.

Trial drilling and assessment

A total of 16 trials were run between mid-July and the end of September. All growers conducted at least one trial although some growers ran up to 3 trials during the season. Drill dates for early sowing tended to be in mid-July and then second sowing in mid-August.

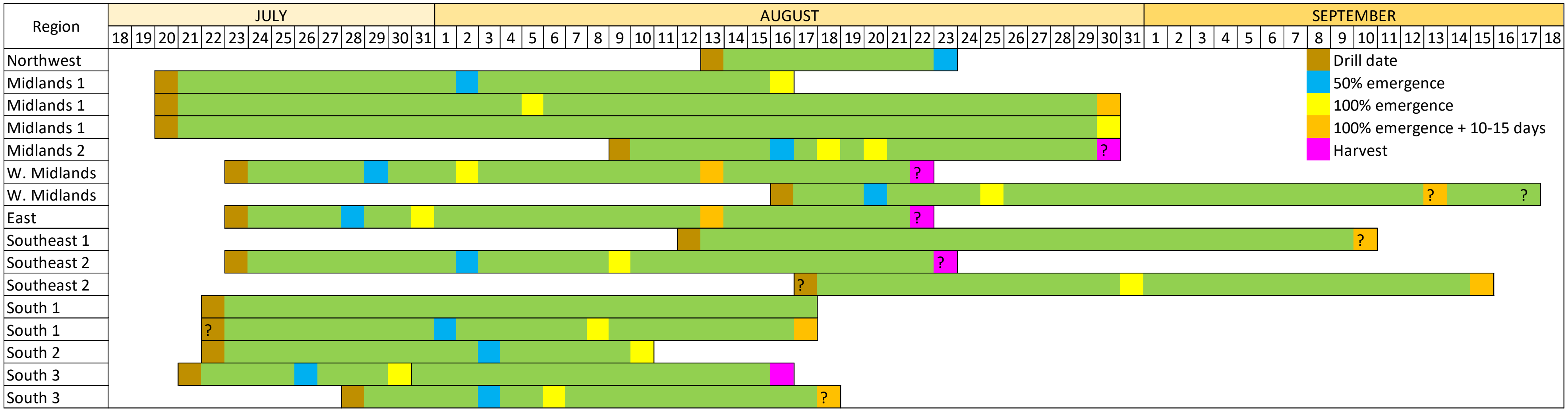

Figure 6. Trial timings for 16 trials run by 9 growers across 10 sites with assessments at different developmental timepoints in 2021.

Damping-off

Overall the level of damping-off observed in all trials was low in 2021, with the majority of observations below 10% plants affected, including for the untreated control. The cause of damping-off was not confirmed. It is unlikely that observed differences between treatments would have been statistically significant from the untreated control. The following comments should be considered noting the low risk conditions for spinach damping-off in these trials. At the first assessment AHDB 9848, AHDB 9763, AHDB 9734 and priming all performed best relative to the untreated control. Across all time points the best overall performer was AHDB 9848. Integral Pro was the worst performer initially but at the last timepoint had a comparable level of damping-off to the untreated control. AHDB 9732 had a similar profile to Integral Pro. AHDB 9763 and AHDB 9734 also performed well at the last timepoint.

Table 8. Effect of treatment on average percentage damping-off. Calculated from 3 quadrat counts of healthy and damped off seedlings per plot.

	Treatment	**% damping-off at different assessment time points (% emergence)**		
		50%	**100%**	**100%+10-15 days**
Control	Untreated	8.3	5.4	3
Conventional fungicide	Maxim 480FS	7.4	6.1	7.2
	AHDB 9848	4.4	3.3	1.4
Biological fungicides	Integral Pro	10.8	7.7	4.6
	AHDB 9734	4.5	7.4	4.2
Biostimulants	AHDB 9732	9.4	3.4	4.6
	AHDB 9733	6.3	8	7.3
	AHDB 9763	4.4	7.6	5.2
Physical	Priming	3.8	10.6	7.5

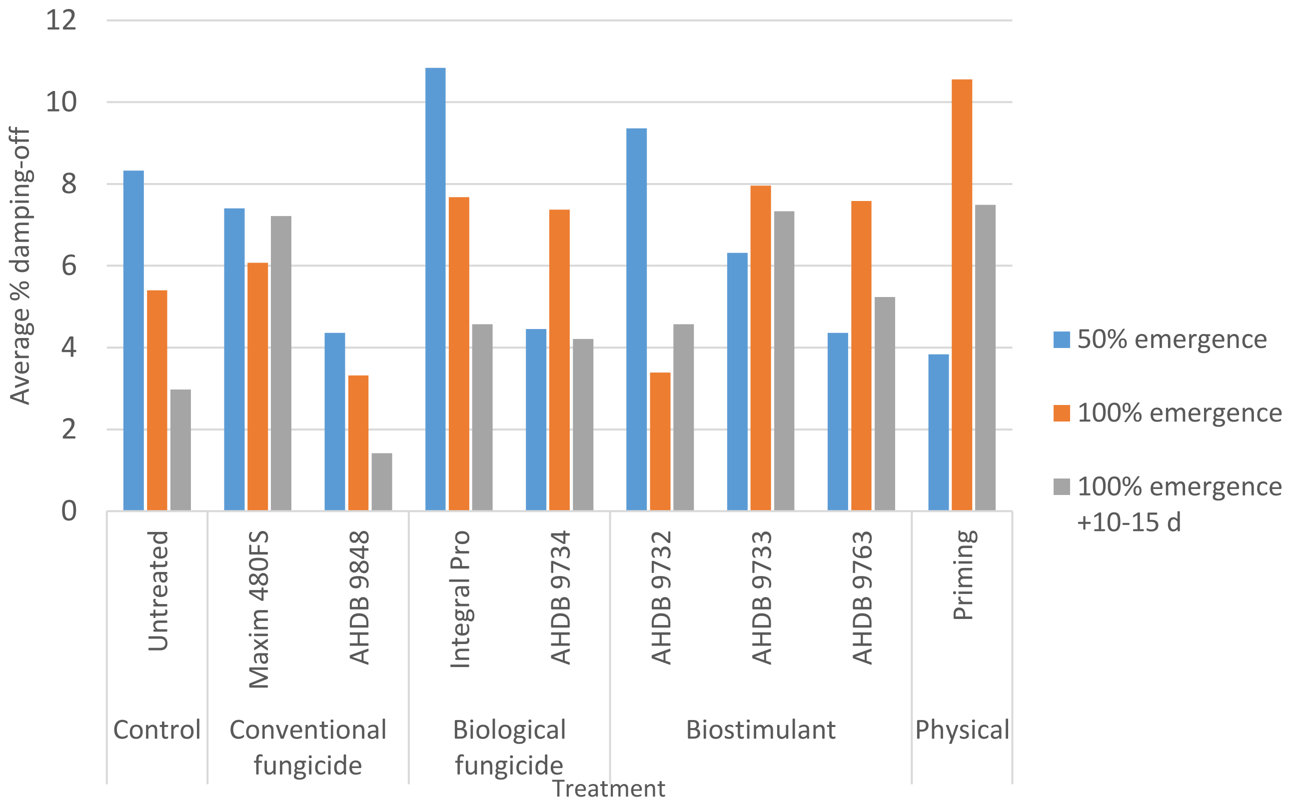

Figure 7. Effect of seed treatment on average percentage damping-off when assessed at different percentage emergence and development timepoints (50% emergence, 100% emergence, 100% emergence + 10-15days + Harvest).

Vigour

Overall, crop vigour was very similar across most treatments with the trend being towards greatest vigour at the first timepoint of 50% emergence. It is unlikely that there would be statistical differences between the treatments. Comments on data trends are as follows: AHDB 9732 and AHDB 9848 were comparable or better to the untreated control at 50% emergence. AHDB 9732 then continued to be the best overall performer across all timepoints outperforming the untreated. AHDB 9734, AHDB 9763 and AHDB 9848 were also all as good as the untreated with very similar profiles through time. Maxim 480FS and AHDB 9733 had comparably good performance at 50% emergence but vigour was reduced compared with other treatments over time and these were the worse overall performers.

Table 9. Effect of treatment on average vigour, scored on 0-10 scale with 5 representing average vigour across all trials.

	Treatment	Crop vigour at different assessment time points (% emergence)			
		50%	100%	100% + 10-15d	Harvest
Control	Untreated	6.4	6.2	6.4	5.5
Conventional fungicides	Maxim 480FS	5.7	4.3	4.8	4.8
	AHDB 9848	6.4	6.4	6.6	5.7
Biological fungicides	Integral Pro	5.9	5	5.9	5.2
	AHDB 9734	5.9	4.9	5.3	5.5
Biostimulants	AHDB 9732	6.7	6.1	6.9	6.5
	AHDB 9733	6	4.8	5.1	4.7
	AHDB 9763	6.1	5.6	5.8	5.4
Physical	Priming	6.3	5.9	6.2	5

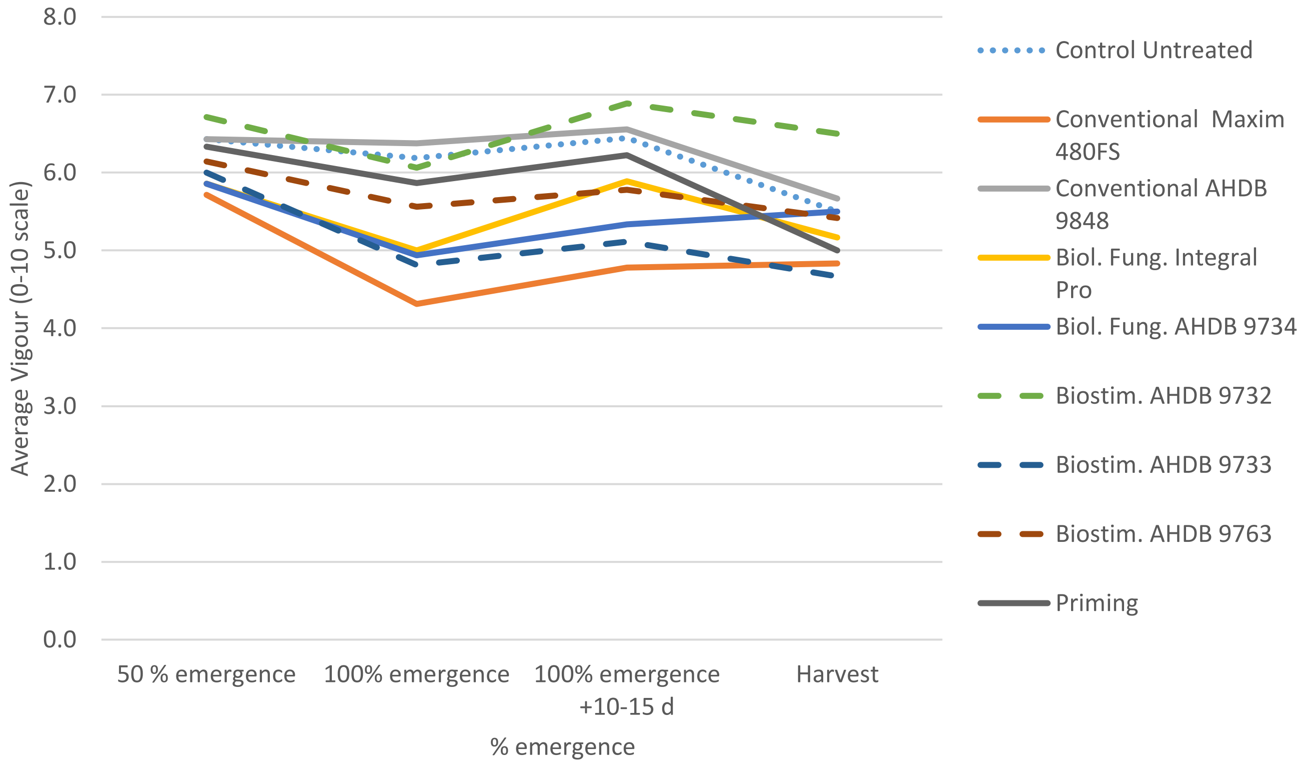

Figure 8. Effect of seed treatment on average vigour of crops (scored 0-10; 5 is average, 10 is most vigorous) when assessed at different percentage emergence and development timepoints (50% emergence, 100% emergence, 100% emergence + 10-15 days, Harvest). Dotted line is the untreated control. Full lines are conventional products or physical treatment i.e., priming. Dashed lines are biostimulants.

Ground cover

There were more marked treatment differences for this parameter. Maxim and AHDB 9733 were consistently the worst performers. Only AHDB 9848 (conventional fungicide) and priming gave higher results than the untreated control. AHDB 9732, a biostimulant, was the best of the biological products tested, with AHDB 9734, a biological fungicide close behind, but both gave less % cover than the untreated control.

Table 10. Effect of treatment on average cover, scored as percentage of whole plot at emergence assessment timepoints across all trials.

	Treatment	**% ground cover at different assessment time points (% emergence)**			
		50%	**100%**	**100% +10-15 d**	**Harvest**
Control	Untreated	37.9	50.8	73.3	91.4
Conventional fungicides	Maxim 480FS	17.1	26.7	48.1	60.8
	AHDB 9848	43.6	52.5	77.2	95.0
Biological fungicides	Integral Pro	25.0	32.1	57.8	67.5
	AHDB 9734	25.7	34.2	58.9	79.2
Biostimulants	AHDB 9732	37.1	45.0	79.4	83.3
	AHDB 9733	22.1	32.7	44.4	65.0
	AHDB 9763	23.6	35.0	60.0	75.0
Physical	Priming	36.7	42.9	69.4	93.3

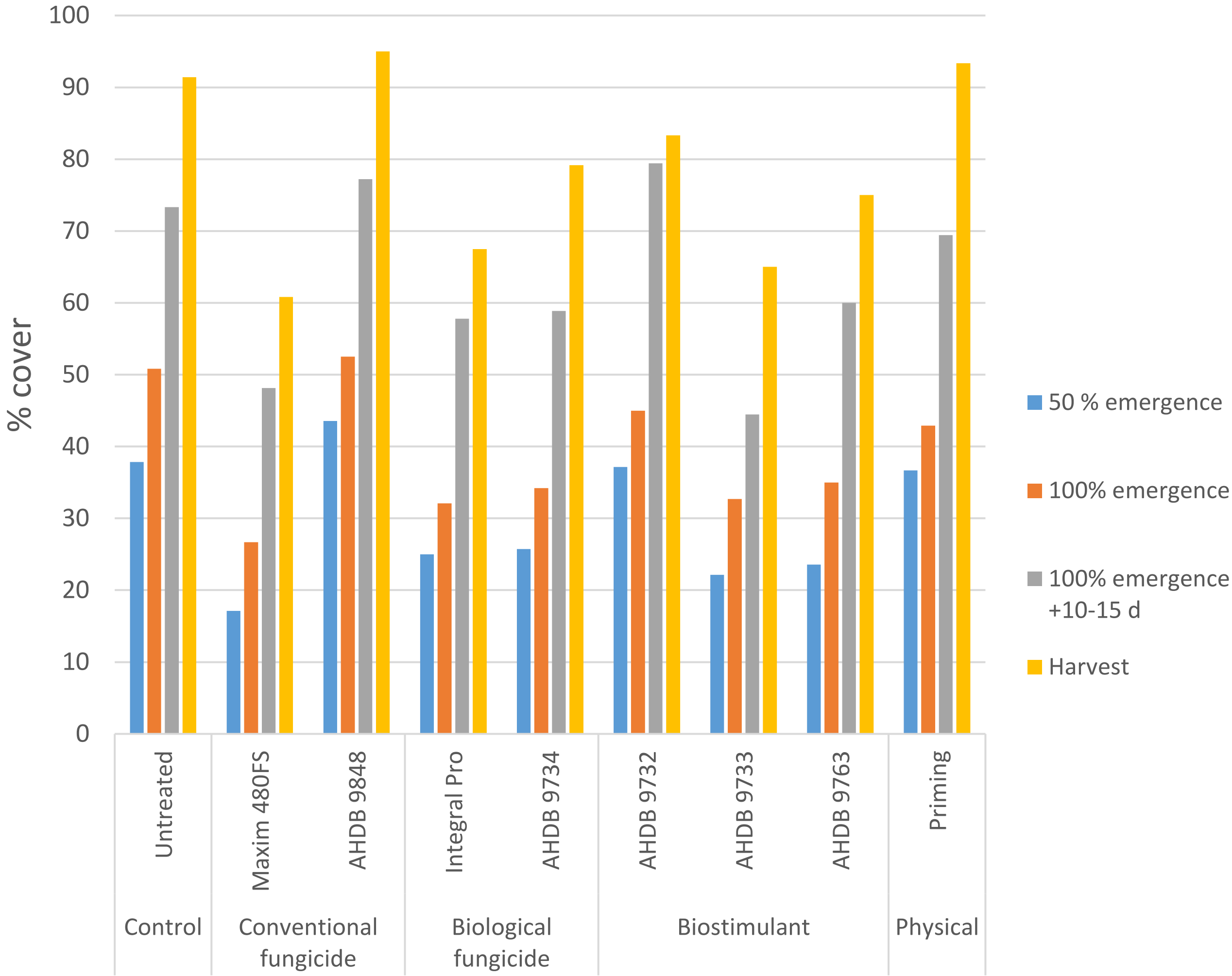

Figure 9. Effect of seed treatment on average cover of crops when assessed at different percentage emergence and development timepoints (50% emergence, 100% emergence, 100% emergence + 10-15days, Harvest).

Phytotoxicity

The greatest effect was seen in all treatments at the 100% emergence + 10-15 days assessment timepoint, with a similar pattern tracked for all treatments. The untreated control had an average score of 4.7 at 100%+10-15 d. Maxim 480FS had the greatest impact at 100% + 10-15d with average score of 3.7. AHDB 9733, priming and the untreated control all had an average score of between 4 and 5 at the 100% +10-15 d timepoint. AHDB 9732, AHDB 9734, AHDB 9763, AHDB 9848 and Integral Pro had average scores between 5.8 and 7.2.

Table 11. Effect of treatment on average phytotoxicity across all trials. Scored on 0-10 scale with 10 = no damage.

	Treatment	Phytotoxicity score at different assessment time points (% emergence)			
		50%	100%	100% +10-15 d	Harvest
Control	Untreated	6.8	6.9	4.7	6.4
Conventional fungicides	Maxim 480FS	5.3	5.5	3.7	5.6
	AHDB 9848	7.3	7.1	7.2	6.8
Biological fungicides	Integral Pro	6.5	6.4	6.1	6.6
	AHDB 9734	6.3	6.2	6.1	6.4
Biostimulants	AHDB 9732	7.3	7	6	7
	AHDB 9733	6.3	6.4	4.6	6
	AHDB 9763	6.5	6.7	5.9	6.8
Physical	Priming	7.6	6.6	4.9	6.6

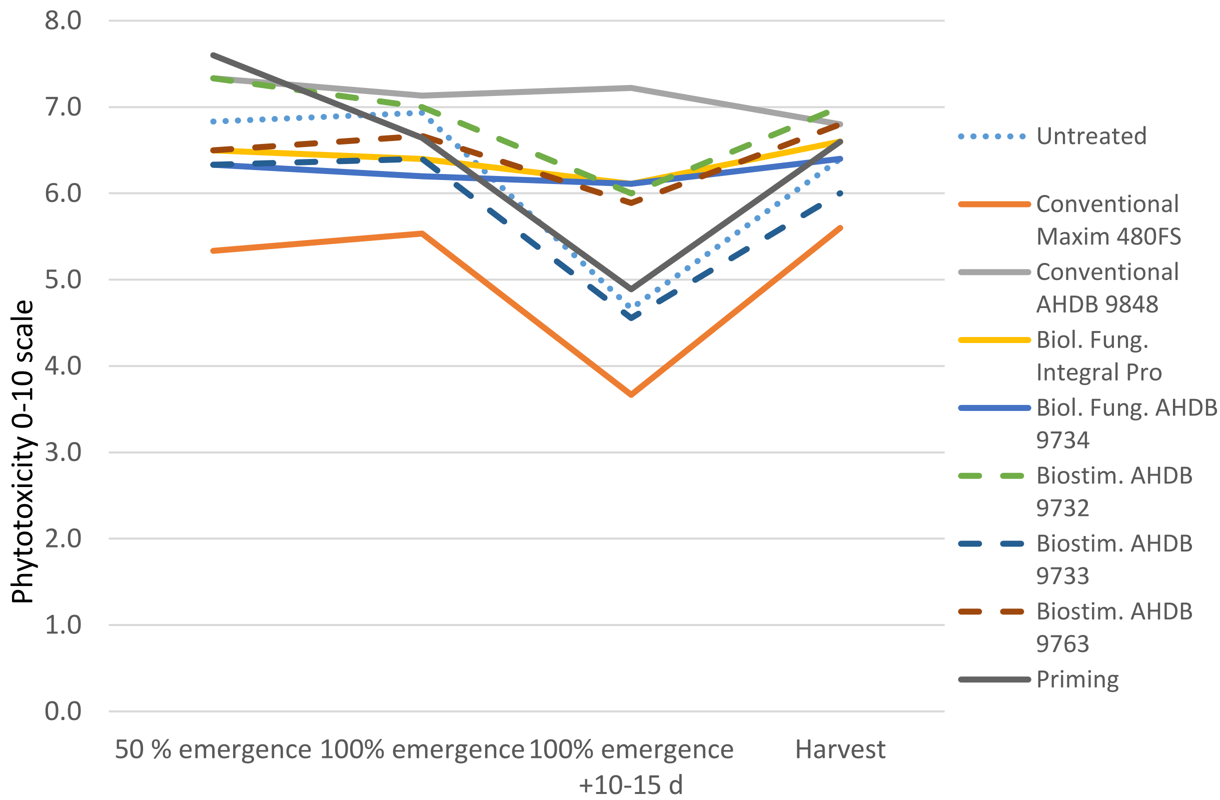

Figure 10. Average phytotoxicity of plots measure on 0-10 scale. 0=complete kill, 10=no damage).

Grower comments on treatment performance

The comments made by growers when collated and assigned ‘traffic lights’, generally aligned well with the crop performance data. Priming, AHDB 9732, AHDB 9848 and the untreated control generally had mostly positive comments. AHDB 9734, Integral Pro and AHDB 9763 were intermediate with a mixture of positive and negative comments. Maxim 480 FS and AHDB 9733 both had overwhelmingly negative comments.

Yields for the treatments at the one site harvested are given in Appendix A. The primed seed yielded 1.1 kg/m and the highest yields were AHDB 9848 (conventional) 3 kg/m and AHDB 9732 (biostimulant).

Table 12. Comments from growers. For individual comments see Appendix A.

Product type	Treatment	Comment summary
Control	Untreated	Mostly positive
Conventional	Maxim 480 FS	All poor
	AHDB 9848	Mostly positive
Biological fungicide	Integral Pro	Mostly poor, some positive
	AHDB 9734	Mostly poor, some positive
Biostimulant	AHDB 9732	Mostly positive
	AHDB 9733	All poor
	AHDB 9763	Mostly poor, some positive
Physical	Priming	Mostly positive

2) Bioassay trials

The overall germination (expressed as the number of germinating seeds as a percentage of total sown) was rather low in experiment 1 (lower inoculum level) ranging from 66.7 to 72.8% (Figure 11). The germination of the untreated control was 69.4%. Very little damping-off disease was observed in inoculated treatments. The lowest level of germination was 60.7% (Maxim 480 FS) and the highest germination percentages were both greater than the untreated controls at 72.8 and 71.1% respectively (AHDB 9763, AHDB 9848, AHDB 9733), although none of these differences were significant at P = 0.05 (Figure 12). Percentage survival to the first true leaf was generally very high in all treatments with the majority of treatments comparable to their relevant untreated control. However, AHDB 9848 and priming both exceeded the untreated control suggesting slight stimulation, again, these differences were not statistically significant.

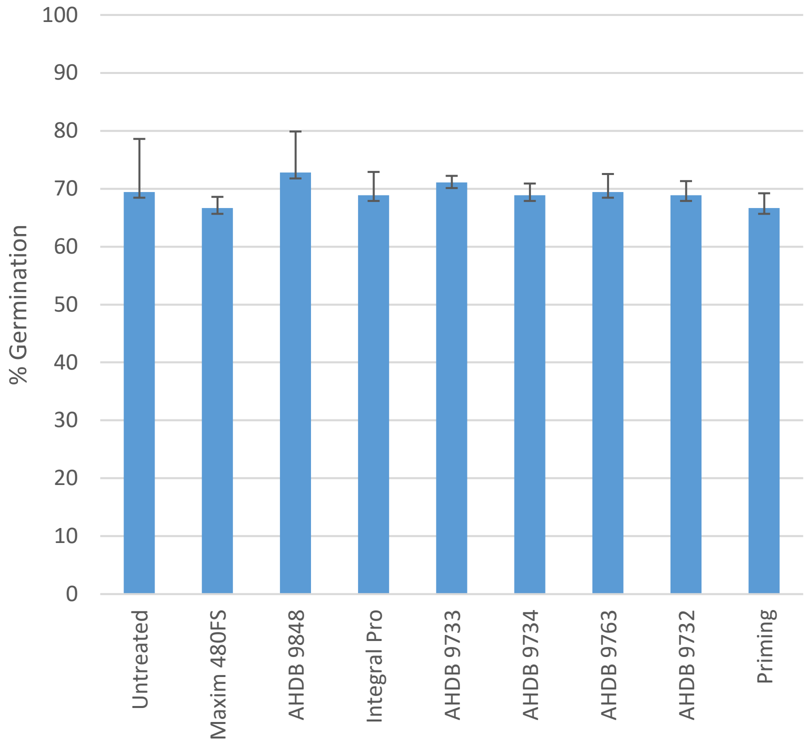

Figure 11. Experiment 1, comparison effects of seed treatments on percentage germination in uninoculated treatments (bars are standard errors of the means of three counts).

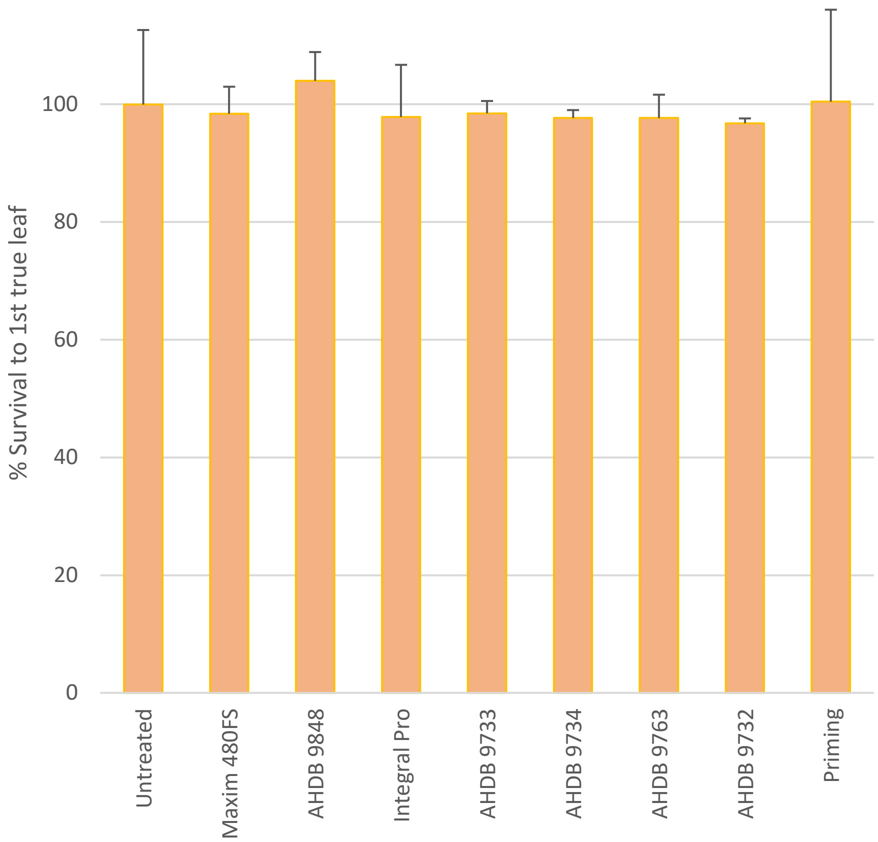

Figure 12. Experiment 1 comparison between seed treatments of germination (survival to emergence of first true leaves) of inoculated treatments as a percentage of their relevant uninoculated controls (bars are standard errors of the means of three counts) .

The *Pythium* inoculum concentration was increased for the second experiment (Experiment 1: 1.6 x 10^3 CFU g^{-1}, Experiment 2: 2.2 x 10^3 CFU g^{-1}). Although a relatively modest increase in inoculum there was dramatically increased level of damping-off disease. AHDB 9848 treatment resulted in the highest level of seedling emergence at almost 21% of uninoculated control (Figure 13). However, emerged seedlings were still susceptible to post-emergence damping-off and the highest % survival (i.e., % seedlings reaching full emergence of their first true leaves) was only 7.2% (AHDB 9733-treated, Figure 13), with AHDB 9848 treated seedlings reduced to 5.2% survival after post-emergence disease. The absolute germination rate was marginally higher in this second experiment, ranging from 78.3 to 88.9% (Figure 14). The various seed treatments did not seem to have much impact on germination relative to untreated controls, with germination varying between 94 and >100% and apparent very slight stimulation from priming (treatment no 2), AHDB 9848 (5), AHDB 9732 (6) and AHDB 9733 (9) treatments (Figure 14).

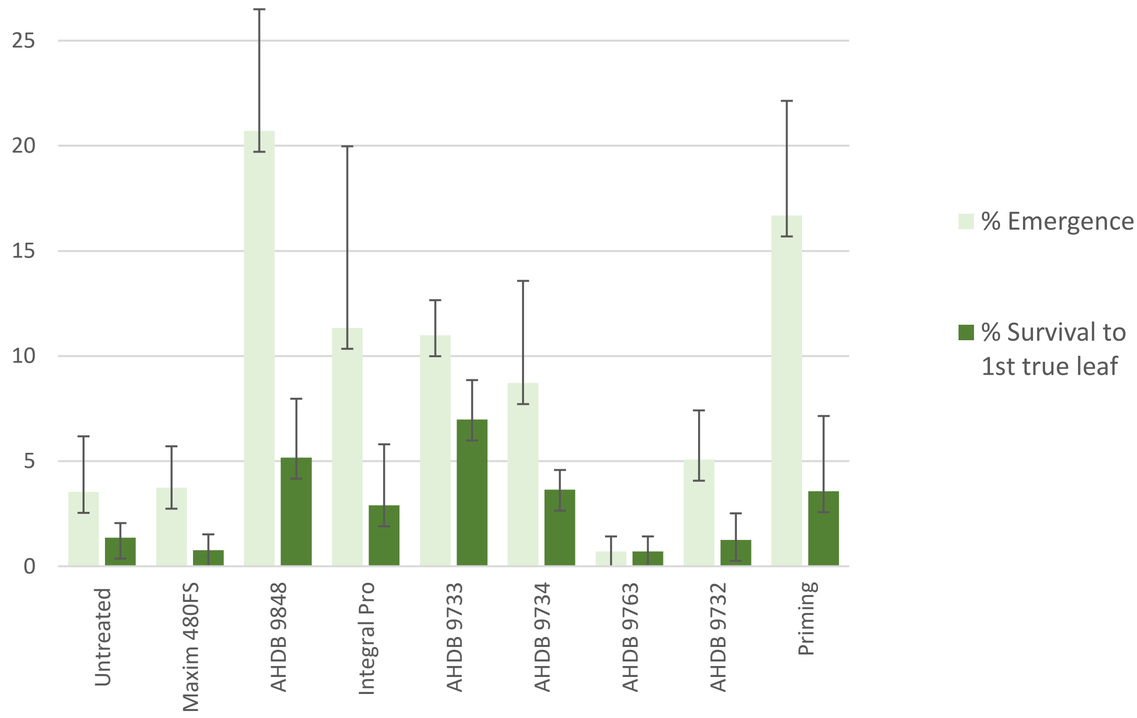

Figure 13. Experiment 2 comparisons between seed treatments of % emergence and of germination (survival to emergence of first true leaves) of inoculated treatments as a percentage of uninoculated controls (bars are standard errors of the means of three counts).

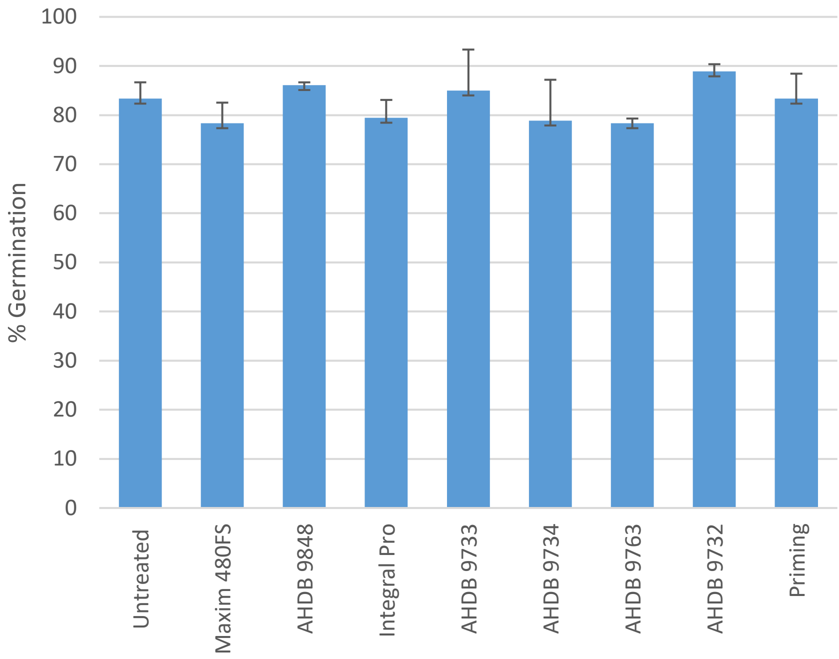

Figure 14. Experiment 2, comparison effects of seed treatments on percentage germination in uninoculated treatments (bars are standard errors of the means of three counts).

Figure 15. Treatments 7 (Maxim 480 FS) (left) and 8 (Integral Pro) (right) from experiment 1. Trays on the left (green labels) are uninoculated controls, those on the right (pink labels) are inoculated trays.

Figure 16. Experiment 2 showing all treatments. Pink labels indicate inoculated trays and green labels uninoculated controls. The sparse levels of emergence and survival clearly indicate the inoculated trays.

Discussion

Since 2020, authorisations have been withdrawn for thiram seed treatment and for Apron XL (metalaxyl-M) as a seed treatment on outdoor drilled crops. The impact of the loss of treatments against damping-off was felt keenly by the industry as significant losses were experienced by spinach growers in 2020, with a total loss of £1.3M. This figure doesn't include the cost to buy in product to cover the losses so the actual economic impact was greater. In this 'bad year' for damping-off in 2020 there were 10-90% average crop losses across fields in the industry (Source: pers. comm. Liz Johnson re: spinach growers survey). In 2021 damping-off incidence was relatively low compared to the previous 2020 season. The potential for such high losses highlighted the need to test viable alternatives for thiram and metalaxyl-M. This grower-led trial provided some valuable insights into the effectiveness of the trial seed treatments on crop performance. The trial was un-replicated so statistical analysis was not possible. Consequently, it is important to note that the data and conclusions drawn in this report represent trends, rather than statistically significant differences between treatments.

Field trials

It is already known that different treatments may have different efficacy against the different damping-off pathogens. Some field sampling was done in 2020 (pers. comm. Liz Johnson) and *Pythium*, *Rhizoctonia* and *Fusarium* species were all detected in different growers' fields. Unfortunately, data is not available to pinpoint where these occurrences were, whether there were coinfections, or any quantification of pathogen load or pathogenicity.

In the 2021 field trials, growers were asked to use trial sites that had been previously affected by damping-off. However, soil analysis from individual growers and their trial sites was not available in order to identify which pathogens were present and connect this with treatment performance across different sites.

The standard *in vitro* germination tests conducted by Elsoms Seeds Ltd. showed that while the germination levels were generally high, all treatments did have some impact on germination. Despite this impact the germination levels remained within a tolerable level that could be offset if a treatment proved to effective in protection against disease.

For field trials, growers were asked to drill as they usually would. This introduced some variability into the data between trials conducted by different growers. At least one grower said that the trial was drilled too deep which would have had an impact on emergence even without the different treatments applied.

In the field trials, the initial assessment at 50% emergence is perhaps the most important observation. A good start to the crop is essential for a successful growing year. The longer the time to 50% emergence the more susceptible the crop is to damage as it takes longer to get established and leaves the young seedlings vulnerable to attack by damping-off pathogens. In terms of damping-off and vigour AHDB 9848 (conventional fungicide), AHDB 9763 (biostimulant) and AHDB 9734 (biological fungicide) performed the best at this initial assessment point. Baby leaf spinach is a fast growing crop and can be ready in 21 days. Delayed emergence or reduced vigour can have a negative impact on yield when it comes to harvest. Good establishment of crops is advantageous to make the crop more resilient to pathogen attack later in the crop's growth cycle. The field trials found that the biostimulant AHDB 9732 had comparatively good vigour across all time points, which may help the crop.

One drawback of the experimental design was that the observations in the field trial were taken at 50% emergence as an average across the trial and all treatments were assessed simultaneously. It wasn't part of the experimental design to assess the time taken to 50% emergence for each individual treatment. However, this would be a potentially interesting metric to study more closely in a future trial to see the differences between treatments in terms of speed of establishment.

The phytotoxicity data presents a few issues. On the 0-10 scale used the economically marketable score/feasible score was set at 8. None of the treatments tested appeared to perform well on this scale. This metric appears to be open to some interpretation and may have been assessed differently by different growers. Consequently, caution is required before drawing any conclusions from this data. Typically, a phytotoxicity score in a herbicide trial is associated with visible scorching of leaves. However, in this trial the phytotoxic effects that were reported tended to be more associated with delay of the crop or deformation of leaves. It would not be expected to observe phytotoxicity in the untreated control, yet this received a score representing less than the perceived optimum healthy growth, alongside some of the treatments. In future trials this metric could perhaps be better defined and the method modified to ask growers to compare directly relative to the untreated and then assign a score.

Yield data was only collected from one trial. Growers in other trials reported that the crop was too thin to harvest at all. Despite the lack of data. The one trial that did report yields appeared to follow the same trends for treatment performance.

One of the most interesting things in this trial was the grower comments. This was analysed in a qualitative way but very much underpinned that the grower experience of growing the crop and their observations are extremely valuable alongside data collection. Their comments aligned very well with the data.

Bioassay

The bioassay experiments included a single isolate of *Pythium ultimum* from one of the 2021 trial sites as a representative damping-off pathogen. The bioassay work highlighted that the inoculum load in the soil is critical to disease development and a relatively small difference in concentration can result in a dramatic difference in disease incidence. In experiment 1 the inoculum load did not appear to be high enough to differentiate between treatments. In experiment 2 the disease incidence was much greater. Differences can be seen in emergence but the overall numbers were very low and the survival to first true leaf was also much lower than in field trials, indicating that the higher rate of inoculum may have been too high and could have potentially masked some of the differences between treatments. Despite this, some trends were seen.

Results across *in vitro*, field and bioassay testing

Across both field trials and bioassays, AHDB 9848 appeared to perform the best in terms of emergence, although survival to first true leaf for this treatment in the bioassay was very low. It was slightly higher than others and not significantly different from other treatments. In contrast to the field trials, AHDB 9733 appeared to perform better than some of the other treatments in the bioassay. There appeared to be slight biostimulation from treatments AHDB 9763, AHDB 9848, AHDB 9733 in the bioassays. Maxim 480 FS performed poorly across both trials.

Conclusions

- This collaborative study approach by experienced growers across a range of locations proved to result in a successful and valuable trial that shows the value of grower engagement to find solutions.
- The results (not statistically analysed) could help to guide growers and agronomists when considering management options for spinach damping-off.
- Understanding the pathogen profile of a trial area may help inform which treatments will be the most impactful to use and bring about the best result.
- AHDB 9848 was the best conventional fungicide treatment in both field trial and bioassay.
- Under conditions that were low risk for damping-off, AHDB 9732 was the best biostimulant and the increase in vigour it seemed to induce may have helped seedling survival. However, it appeared to be less effective at higher inoculum levels in the bioassay.
- Priming is not currently a practical or cost-effective commercial solution.
- Grower comments aligned well with the data collected.
- Bioassay work found no discernible adverse impact of treatments on seed germination but showed that in situations of high inoculum density (*P. ultimum*) even the more promising treatments could be overcome by the pathogens.
- Growers should use all tools available to them (e.g,. rotation, cover crops etc.) to prepare their soil to reduce pathogens loads when drilling crops, even if seed treatments are used.

Knowledge and Technology Transfer

This work was/will be presented at the following:

British Leafy Salads Association newsletter – Interim update, Autumn 2021

British Leafy Salads Association newsletter – Final report, Winter 2021

British Leafy Salal Association R&D Webinar – 3rd March 2022

Brassica and Leafy Salad conference on the 25th October 2022, Peterborough, UK. – Presentation

References

Hunter, PJ, Petch, GM, Calvo-Bado, LA, Pettitt, TR, Parsons, NR, Morgan, JAW, Whipps, JM. (2006). Differences in Microbial Activity and Microbial Populations of Peat Associated with Suppression of Damping-Off Disease Caused by *Pythium sylvaticum*. Applied and Environmental Microbiology 72:6452-6460.

Pettitt, TR, Wainwright, MF, Wakeham, AJ, White, JG. (2011). A simple detached leaf assay provides rapid and inexpensive determination of pathogenicity of *Pythium* isolates to 'all year round' (AYR) chrysanthemum roots. Plant Pathology 60:946–956.

Appendices

Appendix A. Grower comments on demonstration plot treatment trials

Below are comments from growers on each of the treatments at difference timepoints. Each line represents a different grower or trial, but the details of which grower or trial the comments came from have not been included to remain anonymous.

Treatment	Emergence for analysis (grouped)	Comments
AHDB 9734	50	variable results - higher levels of damping-off
		even damping of in plot
		variability in seedlings, signs of damping-off
		Very Poor
		Poor
	100	some yellowing
		Worst
		v even across beds
		new seedlings emerged
		Best of three treatments in plot
		Considerable losses but not the worst
		Significant losses
	100+10-15d	No further symptoms of damping-off
		Emergence suppressed.
		growth not uniform at all
		Poor
	Harvest	best established of treatments in plot
		2.1kg/lm
Integral Pro	50	variable results - higher levels of damping-off

		very few seedlings emerged
		Very poor
		Poor, obvious damping-off
	100	much reduced vigour c/w all others - performed badly in this trial
		Excellent emergence
		excellent vigour which seems to kick in later compared with AHDB 9848 - later kick in seems to be a feature of all bios
		Very thin failure
		Significant losses
	100+10-15d	No further symptoms of damping-off
		Emergence suppressed.
		no damping-off visible.
		Poor
	Harvest	variation in leaf shape/size
		Unable to harvest too thin
		2.1kg/lm
Maxim 480 FS	50	visibly worst of all - impaired germination & vigour though levels of actual damping-off were low
		very little emergence in plot and scattered
		Slight damping-off
		no signs of damping-off
		Very poor
		Poor
	100	some yellowing
		Poor
		Worst by far - no damping-off apparent, just poor emergence
		cupped, twisted leaves, worst by far
		slower emergence, lower vigour
		Very thin, failure
		Significant losses
	100+10-15d	No further symptoms of damping-off
		Emergence significantly suppressed. Unacceptable. Worst treatment.
		poor bottom 1/2, better further up bed. Worst of 3 in bed.
		Poor
	Harvest	thinner
		Unable to harvest too thin
		2.3kg/lm
AHDB 9733	50	patches of poor emergence
		uneven emergence of plot

		variability in seedling growth stage, damping-off evident
		Very poor
		Very thin, poor
	100	Slightly better than integral Pro
		Poor seed coverage - lot of seed on surface, possiby affected germination
		even across bed at finish - useful result
		Very thin, failure
		Very poor, failure
	100+10-15	No further symptoms of damping-off
		Emergence suppressed.
		worst of 3 in bed. Where seedlings have grown, vigour is best out of treatments on RHS of bed.
		Very poor
	Harvest	Unable to harvest too thin
		2kg/lm
Primed	50	Good emergence
		fewer seedlings than treatment 1
		Vigorous, good establishment
		Good
	100	Good
		Excellent emergence
		variable growth stages
		Very good
		Good
	100+10-15d	No further symptoms of damping-off
		Generally, low level of pythium.
		no damping-off visible
		Good density
	Harvest	damping-off patch noted
		1.1kg/lm
AHDB 9732	50	lower emergence rate on outside 4-5 rows where seed less well covered
		even emergence of plot
		variable, still some emerging, signs of damping-off, more seedlings emerged than treatments 2,3,6
		Very poor
		Good
	100	Excellent emergence

		somewhat uneven - less vigour than untreated but acceptable result
		Best of three treatments in plot - some yellowing
		Very thin, failure
		Good
	100+10-15	No further symptoms of damping-off
		Emergence suppressed.
		some yellowing visible.
		Good density
	Harvest	more pointed leaves
		Unable to harvest too thin
		3.5kg/lm
AHDB 9763	50	patches of poor emergence
		signs of damping-off
		Very poor
		Very thin, poor
	100	Poor, limited germination
		More vigorous than AHDB 9734 or Panormaix
		again, centre of bed showed greater vigour
		slower growth, 3rd leaf just emerging
		Very thin, failure
		Very poor, failure
	100+10-15d	No further symptoms of damping-off
		Emergence suppressed.
		best of 3 in bed.
		Very poor
	Harvest	Unable to harvest too thin
		2.8kg/lm
AHDB 9848	50	best on intial obs - excellent vigour & emergence
		Good coverage
		variable seedlings
		Delayed emergence but plant numbers appear good
		Very good
	100	Best
		Excellent emergence
		most vigour of all plots - noticably paler at point of harvest
		variable growth stages
		Delayed emergence but good very few losses
		Good
	100+10-15d	No further symptoms of damping-off

		Slightly worse than untreated or primed.
		best of 3 in bed.
		Best in this trial, good density
	Harvest	3kg/lm
Untreated	50	second best on intial obs
		Good emergence
		slower emergence, some seedlings just emerging, signs of damping-off
		Good establishment
		Good
	100	Excellent emergence
		no issues - centre of bed more vigourous, probably caused by cultivations
		more new seedlings emerged, variable growth stages
		Best of three treatments in plot
		Good
		Good
	100+10-15d	No further symptoms of damping-off
		Generally, low level of pythium.
		bad across whole plot, not grown well. Damping-off, yellowing, leggy and stunting. Worst of 3 in bed
		Good density
	Harvest	2kg/lm