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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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1 GROWER SUMMARY

1.1 Headline

- In two experiments, treatment of onion seed with an experimental fungicide formulation eliminated external and internal *B. allii*, even from a seed batch with a high percentage infection. There was no deleterious effect of the treatment on percentage seed germination at either 1 month or 5 months after treatment.
- The effects of hot water treatments on onion seed germination varied according to seed batch health and maturity. The most promising results were obtained when seed was pre-soaked at 20°C for 18 h prior to hot water treatment (45°C) for 30 or 45 min, reducing *B. allii* infection to 0.5% or less with no effect on percentage germination, irrespective of seed batch.
- Of three disinfectants tested as seed treatments, Jet 5 (peroxyacetic acid) was the most effective. Following treatment with 5% or 10% Jet 5 (20 min), no *B. allii* was detected in a seed batch known to contain high levels of the pathogen (external and internal) and there was no reduction in percentage germination.

1.2 Background and expected deliverables

Infected seed is a major source of inoculum for neck rot (*B. allii*) which can lead to significant losses of onions in store if crops are left untreated. Following the withdrawal of Benlate fungicide, the standard industry seed treatment for onion neck rot is now Hy-TL (thiabendazole and thiram), which has had a specific off-label approval since 2002. There is currently pressure from retailers to reduce usage of thiabendazole, and so there are concerns within the onion industry that reliance on this single seed treatment for neck rot may be unsustainable.

The overall objective of the project is to determine the efficacy of a range of seed treatments for the control of onion neck rot that could provide an alternative to Hy-TL (thiabendazole + thiram) for use in onion production.

The specific objectives are to:

1. Finalise seed treatments for evaluation, based on findings of a knowledge review, consultation with the industry and their potential suitability for conventional and organic production.
2. Source a stock of onion seed naturally infected with *B. allii* at a high level and select an appropriate method for determining the incidence of viable infection in replicate sub-samples.
3. Evaluate selected seed treatments for their effects on seed germination and vigour, before and after storage, using standard seed testing protocols.
4. Determine the efficacy of selected treatments in reducing seed-borne inoculum of *B. allii* in naturally infected onion seed, compared with the current standard treatment (Hy-TL).

The project will provide information on both the efficacy and feasibility of a range of seed treatments for onion neck rot, enabling the industry to make an informed decision on viable alternatives for use in onion production. While the project will focus on onion neck rot as a model system, it is anticipated that results will be relevant to broader research on the control of seed-borne diseases on other horticultural crops.

1.3 Summary of the project and main conclusions

1.3.1 Standard methods

- Onion seed batches naturally infested with *B. allii* were sourced from a commercial seed company. Three seed batches from a single cultivar were used in each experiment to ensure that different treatment methods were evaluated against seed with different levels of *B. allii* infection (internal and external); nil, moderate and high.
- Based on published literature, seed batches used for experimental work in this project were tested for the incidence of *B. allii* by surface sterilising and plating onto selective media. For experiments in project year 1, Prune Lactose Yeast Agar amended with streptomycin and erythromycin was used. Subsequently, modified Kritzman's agar was used in order to reduce the incidence of other microbial contaminants that were developing from onion seed and potentially suppressing the growth of *B. allii*.

- A neck rot infection of 10% or more in store can lead to rejection. Previous researchers demonstrated that to achieve a neck rot incidence of less than 10% in store, the incidence of *B. allii* in seed should be 1% or less. This threshold provided a useful baseline for determining the efficacy of seed treatments tested in this project.

1.3.2 Fungicides

- Two experiments to test the efficacy of fungicide seed treatments against *B. allii* gave promising results. The following were tested: Hy-TL (industry standard), Wakil XL (three doses), Raxil, and three doses of an experimental seed treatment formulation (containing a combination of two active ingredients).
- Raxil significantly reduced percentage seed germination in the first experiment and was not included for further testing.
- Wakil XL (10 g per million seeds) was effective against external botrytis (even for a seed batch with high contamination levels) but was less effective against internal botrytis. Lower doses did not provide consistent pathogen kill. Seed germination was not affected.
- In two experiments, the higher dose of the experimental formulation eliminated external and internal *B. allii* from a seed batch with high infection levels (25% external, 5% internal) with no deleterious effects on seed germination, even when treated seed had been stored for 5 months. Further work to determine the effect of this fungicide seed treatment on the subsequent incidence of neck rot in the field would now be warranted.
- All of the fungicide treatments reduced but did not eliminate seed contamination due to other micro-organisms.

1.3.3 Hot water treatment

- In three hot water treatment experiments, the treatment effects on seed germination varied according to seed batch, with the seed batches containing moderate and high levels of *B. allii* being more sensitive to treatment than the botrytis-free seed batch.

- Hot water treatments (45°C) for 30 or 45 min provided effective control of *B. allii* but effects on seed germination were dependent on seed batch health and maturity.
- The most promising results were obtained when seed was pre-soaked at 20°C for 18 h prior to hot water treatment (45°C) for 30 or 45 min. These treatments reduced *B.allii* infection to 0.5% or less with no effect on percentage germination, irrespective of seed batch.
- Microbial contamination on seeds was reduced following treatments at 45 or 50°C but not eliminated.

1.3.4 Disinfectants

- The disinfectants Jet 5, sodium hypochlorite and Vitafect were tested using a range of concentrations and soak durations, for their efficacy in eliminating *B. allii* from onion seed and their effects on onion seed germination.
- As with hot water treatment, seed sensitivity to disinfectant treatment varied with seed batch health and maturity.
- Vitafect appeared promising in a first experiment but in subsequent experiments, effects on *B. allii* and seed germination were inconsistent.
- At concentrations and soak durations required to provide effective control of *B. allii*, sodium hypochlorite had a deleterious effect on seed germination.
- Jet 5 provided the most consistent control of *B. allii* with no deleterious effect on seed germination after either 2% Jet 5 for 6 h, or 10% Jet 5 for 20 min, irrespective of seed batch. No *B. allii* was detected in either of two seed batches following treatment with 5% or 10% Jet 5 for 20 min.

1.4 Financial benefits

The work has highlighted effective seed treatment techniques for onion neck rot that could potentially be scaled-up for commercial use. It is anticipated that the findings could provide the basis for further research on alternative treatments for seed-borne diseases of horticultural crops, to be developed in conjunction with industrial partners.

1.5 Action points for the industry

Discussions are ongoing with the agro-chemical company providing the experimental seed treatment formulation to determine the future availability of this experimental product on vegetable seed.

2 SCIENCE SECTION

2.1 Introduction

Within the vegetable industry, there is increasing interest in alternatives to fungicidal seed treatments for the control of seed-borne diseases, both i) in conventional production, due to fungicide withdrawals and consumer preference for minimal pesticide usage and, ii) in organic systems, where the use of fungicide-treated seed is no longer permitted (EU regulation 2092/91). There is particular concern regarding diseases for which seed represents a major source of inoculum such as onion neck rot (*Botrytis allii,* also known as *B. aclada*), which can lead to significant losses if crops are left untreated. For example, results from a Defra-funded project on the feasibility of organic seed production (project OF0166), showed that neck rot will be the main constraint in production of organic onion seed.

Following the withdrawal of Benlate fungicide, the standard industry seed treatment for onion neck rot is now Hy-TL (thiabendazole and thiram) which has had a specific off-label approval since 2002. There is currently pressure from retailers to reduce use of thiabendazole, and so there are concerns within the onion industry that reliance on this single seed treatment for neck rot control may be unsustainable. There are also fungicide resistance concerns relating to MBC products such as thiabendazole (Gladders *et al*., 1994).

Potential alternatives to fungicide seed treatments include the use of physical methods (e.g. hot water, steam, UV and microwaves), disinfectants, botanical extracts or products, and biological control. For example, recent HDC-funded work (FV 237a) showed the potential for control of celery leaf spot (*Septoria apiicola*) using seed treated with either hot water or Jet 5 (peroxyacetic acid). In addition to non-fungicidal treatments, there may be other fungicides that could provide alternative seed treatments for onion neck rot.

The overall aim of the current project is to determine the efficacy of a range of seed treatments for the control of onion neck rot that could provide an alternative to Hy-TL (thiabendazole + thiram) for use in onion production. The previous report provided a

review of potential methods for control of seed-borne *B. allii* together with results from preliminary seed treatment experiments. This final report describes experiments continuing from studies done in project year 1 to determine the effect of hot water treatment, disinfectants and fungicides on the percentage infection of onion seeds by *B. allii* and percentage germination of onion seed.

2.2 Standard methods

2.2.1 Seed batches

Onion seed batches naturally infested with *B. allii* were sourced from a commercial seed company. Three different seed batches from a single cultivar were used in each experiment to ensure that different treatment methods were evaluated against different levels of *B. allii* infection; nil (Batch 156719), moderate (Batch 156721) and severe (Batch 156720). Results from seed testing by the commercial seed company and ADAS are shown in Tables 1 and 2 below. Seed testing methods used by ADAS are described in Sections 2.2.3 and 2.2.4. Differences in the detection of *B. allii* are likely to be due to differences in testing methods (seed company protocol not disclosed).

Table 1. Seed germination and percentage infection due to *Botrytis allii* in onion seed lots, after deep freezer storage (results from commercial seed company, methods not disclosed)

	Seed germination (%)*			*B. allii* (%)**	
Seed lot	GE	GC	Abn	External	Internal
156719	94	96	1	0	Not detected
156720	84	89	4	100	49
156721	93	96	2	63	10

GE = germination energy
GC = germination capacity
Abn = abnormal
*200 seeds tested
**400 seeds tested

Table 2. Seed germination and percentage infection due to *Botrytis allii* in onion seed, after deep freezer storage (ADAS)

Seed lot	Normal seed germination (%)*	*B. allii* (%) internal**
156719	96	0
156720	93	31
156721	95	2

*200 seeds tested

** 175 seeds tested

2.2.2 Seed storage

Seeds received from the commercial company had been retrieved from deep-freezer storage. The bulk of seed for use during this project was stored at Elsoms seeds Ltd, Spalding, Lincolnshire in controlled environment storage (<10°C, 30% RH). Seed sub-samples for use in laboratory experiments were stored in the refrigerator at ADAS Arthur Rickwood.

2.2.3 Seed germination test

The method used for seed germination testing throughout the project reflects commercial practice and training was provided by staff at Elsoms seeds Ltd. Seed germination boxes were prepared by inserting a pleated filter paper in a clear plastic box, ensuring the filter paper was the correct way up to give 50 pleats. A sheet of filter paper was folded around the filter paper pleat and overlapping on top. Tap water (50 ml) was added to each box and then left for at least 2 hours before adding any seeds. For each seed batch, two rows of 50 seeds were placed into two boxes (200 seeds in total). The lids were replaced and the boxes were incubated at 20°C (8 h light / 16 h dark) in a controlled environment cabinet for 6-8 days. After this time all seeds were assessed and classified into the following categories:

Normal: shoot should be green, with a definite 'elbow'. There should be minimal swelling. There should be sufficient root development to 'balance' the seedling.

Abnormal: abnormalities include:

- Short, thick shoot development
- Poor root development
- No definite elbow
- Seedling bent over or constricted
- Seedling forming a loop or spiral
- Spindly growth

Fresh seed: (unusual for onion) seeds which remain firm and apparently viable at the end of the test are classified as fresh ungerminated seed and are reported separately from the percentage germination.

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, are classified as dead seeds.

If germination was >96%, the test was finished at 7 days. Otherwise, the seedlings were re-assessed at 10 to12 days.

2.2.4 Incidence of Botrytis allii on seed

Serological methods and PCR-based methods for detecting *B. allii*, particularly in onion bulbs (e.g. Linfield *et al*., 1995; Nielsen, 2002) are available. However, for high-incidence pathogens such as *Botrytis allii* that occur in seed samples at levels greater than 1% and which can be detected by testing 200-400 seeds, agar plate tests provide the simplest means for pathogen detection (Maude, 1996). Metcalf (2002) described the advantages and disadvantages of a range of agar media that are used routinely by diagnostic laboratories to quantify incidence of *B. allii* in onion seed. Seed testing for *B. allii* is done by surface sterilising seed in sodium hypochlorite (NaOCl) before rinsing and incubating on a laboratory agar which allows *B. allii* to grow and produce spores that can be identified by microscope. The concentration and duration of surface sterilisation treatment is an important part of the test since too much sterilisation may eradicate *B. allii,* but insufficient sterilisation may result in overgrowth of fungal contaminants.

Based on methods described by Metcalf (2002), seed batches to be used for experimental work in this project were initially tested for the incidence of *B. allii* by surface sterilising and plating onto half strength Lactic Acid Potato Dextrose Agar (LPDA) (Appendix 1). Subsequently, Prune Lactose Yeast Agar amended with streptomycin and erythromycin (PLYSE) (Appendix 1) was used for seed plating, as it is more widely accepted for the determination of botrytis incidence. In project year 2, however, seeds were plated onto Botrytis Selective Media (BSM) (Appendix 1; modified from Kritzman & Netzer, 1978), another widely used agar for determination of botrytis incidence. This modification was made in order to reduce the growth of microbial contaminants from onion seed (e.g. *Mucor* sp.), that were potentially suppressing the growth of *B. allii*. The following method was used:

Composite seed samples of batches to be tested were immersed in 3% sodium hypochlorite in individual beakers for 1 minute. After this time the liquid was decanted off through muslin leaving the seeds to be rinsed in two changes of 100 ml sterile distilled water for 1 minute each. Seeds were dried on sterile filter paper before being plated out onto the selected agar. Tweezers were dipped in 90% ethanol and flame sterilised every five seeds before dipping in sterile distilled water. Excess water on the tweezer tips was absorbed by pressing the tweezer tips onto sterile filter paper. Seeds were then individually placed onto the selected agar, 25 seeds per plate. For each seed batch and treatment, 400 surface sterilised seeds and 400 non-sterilised seeds were plated out to determine the incidence of internal and external botrytis, respectively. All seeds were incubated at approximately 20°C for 5-10 days. After this time all seeds were examined for the presence of *B. allii*.

2.2.5 Identification of Botrytis allii

Colonies of *B. allii* were identified as follows: *B. allii* has characteristic conidiophores, which appear silver-white when lit from above and are carried in small bunches above the surface of the mycelium. The conidiophores of *B. cinerea* look very similar but the mycelium is less compact and growth is more rapid than *B. allii*. Suspect colonies of *B. allii* were sub-cultured on to plates of PDA+S by aseptically transferring small pieces from the leading edge of the colony, for further examination.

Conidia from three colonies identified as *B. allii* were measured and had the following dimensions: length range 7.5 - 10 µm (mean 8.4 µm); width range 3.8 – 5.6 µm (mean 5.0 µm). This corresponds with dimensions published for *B. allii* conidia (Ellis & Waller, 1974).

2.2.6 Statistical analyses

Data were analysed statistically using analysis of variance (ANOVA) in Genstat with treatment comparisons examined using SEDs (standard error of the difference between means).

2.3 Fungicide treatment

2.3.1 Objectives

i) To determine the effect of selected fungicide treatments on percentage germination of onion seed after 5 months storage.

ii) To re-test seed from selected treatments from a previous fungicide experiment (project year 1) using a different selective agar media.

iii) To determine the effect of fungicide treatments on percentage infection of onion seeds by *Botrytis allii* and percentage germination of onion seed.

2.3.2 Methods

Experiment 1

Onion seeds that had received fungicide treatment in project year 1 (see Annual report) were subsequently stored in sealed Petri dishes in a refrigerator. After 5 months, the percentage germination of seeds from selected treatments (Table 3) was re-tested. Germination tests were done using four replicates of 50 seeds (a total of 200 seeds) from each seed batch (156719, 156720 and 156721) and assessed after 12-14 days (Section 2.2.3).

Experiment 2

In a first fungicide seed treatment experiment (Year 1 Annual Report), promising results were obtained with a coded formulation (containing two active ingredients). However, it was noted that growth of other micro-organisms from the onion seed partially suppressed development of *B. allii* in the untreated control, making it difficult to compare treatments based on the percentage seeds with external *B. allii* infection. In this experiment, two treatments from the year 1 experiment, the untreated control and coded formulation A (Table 3), were re-tested on BSM agar instead of Prune Lactose Yeast Agar. For these two treatments, the incidence of *B. allii* was tested for 400 non-surface sterilised and 400 surface sterilised seeds from seed batches 156720 and 1567221 on BSM agar (Section 2.2.4).

Experiment 3

The coded fungicide formulation used in year 1 was re-tested (three doses), together with Wakil XL (three doses), Hy-TL (industry standard) and an untreated control. Raxil was not re-tested because of its deleterious effect on seed germination.

A 200 g composite sample of each seed batch (156719, 156720 and 156721) was subjected to each of the fungicide treatments shown in Table 4. Fungicides were applied as a fluidised-bed film coating at Warwick HRI, Wellesbourne (Dr A. Jukes, pers. comm.). Seeds were left to air-dry at ambient temperature before dispatching to ADAS Arthur Rickwood for botrytis testing.

For each fungicide treatment, seed germination tests were done on four replicates of 50 seeds (a total of 200 seeds) from each seed batch and assessed after 12-14 days (Section 2.2.3). The incidence of *B. allii* was tested for 400 non-surface sterilised and 400 surface sterilised seeds from seed batches 156720 and 1567221 on BSM agar (Section 2.2.4).

Seeds from treatments 6-8 (coded formulations) were sent to the supplier of the experimental fungicide to check for fungicide loading by HPLC.

Table 3. Fungicide treatments applied to onion seeds that were re-tested for percentage germination after 5 months storage (Experiment 1)

Product	Active ingredient	Dose	Product dose per 200g onion seed sample*
1. Untreated control	-	-	-
2. Hy-TL	225 g/L thiabendazole + 300 g/L thiram	9 ml product per kg seed	1.8 ml
3. Wakil XL**	50 g/kg fludioxonil 100 g/kg cymoxanil + 175 g/kg metalaxyl-M	5 g product per million seeds	0.25 g
4. Coded formulations*** Dose A	a.i. 1:50 g/L a.i. 2:50 g/L	Code 082: n Code 114: n	Code 082: n Code 114: n
5. Coded formulations*** Dose B	a.i. 1:50 g/L a.i. 2:50 g/L	Code 082: 0.5 x n Code 114: 0.5 x n	Code 082: 0.5 x n Code 114: 0.5 x n
6. Coded formulations*** Dose C	a.i. 1:50 g/L a.i. 2:50 g/L	Code 082: 4 x n Code 114: 4 x n	Code 082: 4 x n Code 114: 4 x n

Notes:

* Average of 2500 seeds per 10 g (pers. comm. R. Cook, Elsoms Seeds Ltd)

** Based on SOLA 1191/02 for carrot and parsnip seed (used under experimental approval on onion seed)

*** Combination of two experimental formulations each containing a different active ingredient; an inert polymer (peridiam red) was also applied to treatments 4, 5 and 6 at the rate of 10 ml/1 kg seed.

Table 4. Fungicide treatments tested against *Botrytis allii* on onion seed (Experiment 3)

Product	Active ingredient	Dose	Product dose per 200g onion seed sample*
1. Untreated control	-	-	-
2. Hy-TL	225 g/L thiabendazole + 300 g/L thiram	9 ml product per kg seed	1.8 ml
3. Wakil XL** Dose A	50 g/kg fludioxonil 100 g/kg cymoxanil + 175 g/kg metalaxyl-M	5 g product per million seeds	0.25 g
5. Wakil XL** Dose B	50 g/kg fludioxonil 100 g/kg cymoxanil + 175 g/kg metalaxyl-M	7.5 g product per million seeds	0.375 g
6. Wakil XL** Dose C	50 g/kg fludioxonil 100 g/kg cymoxanil + 175 g/kg metalaxyl-M	10.0 g product per million seeds	0.5 g
6. Coded formulations*** Dose A	a.i. 1:50 g/L a.i. 2:50 g/L	Code 082: n Code 114: n	Code 082: n Code 114: n
7. Coded formulations*** Dose B	a.i. 1:50 g/L a.i. 2:50 g/L	Code 082: 0.5 x n Code 114: 0.5 x n	Code 082: 0.5 x n Code 114: 0.5 x n
8. Coded formulations*** Dose C	a.i. 1:50 g/L a.i. 2:50 g/L	Code 082: 4 x n Code 114: 4 x n	Code 082: 4 x n Code 114: 4 x n

Notes:

* Average of 2500 seeds per 10 g (pers. comm. R. Cook, Elsoms Seeds Ltd)

** Based on SOLA 1191/02 for carrot and parsnip seed for treatment 3 and higher rates for treatments 4 and 5 (used under experimental approval on onion seed)

*** Combination of two experimental formulations each containing a different active ingredient.

2.3.3 Results and discussion

Experiment 1

Although the mean percentage germination for each treatment decreased over time (Table 5), there was no significant effect of fungicide treatment on percentage normal germination when seeds were stored for 5 months after fungicide treatment.

As in year 1, there was a significant effect of seed batch on germination with Batch 156719 (botrytis-free) giving higher percentage germination levels.

Table 5. Effect of fungicide treatment on percentage of onion seeds with normal germination after 5 months of storage

Fungicide treatment	Mean % onion seed germination 1 month after fungicide treatment	% onion seed germination 5 months after fungicide treatment			
		Batch 156719	Batch 156720	Batch 156721	Means
1. Untreated control	94.0	89.5	83.0	83.5	85.3
2. Hy-TL	97.8	91.0	83.0	87.0	87.0
3. Wakil XL	97.3	89.0	81.0	87.5	85.8
4. Coded formulations Dose A	97.2	90.0	81.5	82.5	84.7
5. Coded formulations Dose B	97.8	92.5	87.0	86.0	88.5
6. Coded formulations Dose C	97.7	86.5	86.0	87.5	86.7
Means	97.0	89.8	83.6	85.7	
D.f.					54
S.e.d. (treatment) n.s.					2.0
S.e.d. (seed batch) ***					1.4
S.e.d. (treatment.seed batch) n.s.					3.4

156719 – nil botrytis, 156720 – high botrytis, 156721 – moderate botrytis
n.s. not significant, *** significant at $P<0.001$

Experiment 2

Results from re-testing of selected seed treatments from a fungicide experiment in year 1 using a different selective agar medium, confirmed the high levels of external *B. allii* present on untreated seed of batch 156720. The results also confirmed the ability of coded formulation (dose A) to reduce both internal and external *B. allii* to less than 1% (Table 6).

Table 6. Effect of fungicide treatment on the incidence of *Botrytis allii* in onion seed (re-tested from year 1 experiment on BSM agar)

Fungicide treatment	Internal		External	
	Batch 156720	Batch 156721	Batch 156720	Batch 156721
1. Untreated control	20.8	0.0	90.5	2.5
2. Coded formulation Dose A	0.5	0.0	0.8	0.0

156720 – high botrytis, 156721 – moderate botrytis

Experiment 3

Percentage normal germination varied significantly with seed batch, with batch 156720 (high botrytis incidence) giving lower percentage germination than the other two batches (Table 7). There was no significant effect of fungicide treatment on percentage normal germination (Tables 8 and 9). These results were in agreement with findings from year 1 when neither Wakil XL nor the coded formulation reduced percentage seed germination in comparison with the untreated control at the doses tested.

There was a significant treatment/seed batch interaction effect ($P<0.001$) (as well as main effects) on the incidence of both external and internal *B. allii* (Table 8). For seed batch 156721, levels of both internal and external botrytis detected were low even for the untreated control (0.3% and 1.8% respectively). For seed batch 156720, Hy-TL, the highest dose of Wakil XL and all three doses of the coded formulation reduced external botrytis from 24.8% to 1.8% or less. For the same seed batch, HyTL and the coded formulation (all doses) gave a significant reduction in the incidence of internal botrytis. Wakil XL at the highest dose reduced external botrytis to 0.3% compared to 24.8% in the untreated control, however lower rates were less effective and the fungicide was not so effective for elimination of deep-seated internal botrytis infection. For both seed batches, Dose A (n) of the coded formulation reduced both external and internal botrytis to 1% or less, while dose C (4n) eliminated the fungus.

External microbial contaminants on onion seed batches 156720 and 156721 included *Mucor* sp., *Cladosporium* sp., and *Penicillium* sp, as well as bacteria. All of the treatments significantly reduced the percentage of external microbial contaminants (Table 9). For internal infection by other microorganisms (including *Botrytis cinerea* in batch 156720), only the coded formulation at dose 4n reduced the percentage infection to less than 1%.

The loading of the experimental formulations (treatments 6, 7 and 8) on seed batches was determined by HLPC (Table 10). The majority of applications were in the target dose range as specified by the manufacturer.

Maude & Presly (1977) stated that a neck rot infection of 10% or more in store can lead to rejection. They demonstrated that to achieve a neck rot incidence of less

than 10% in store, the incidence of *B. allii* in seed should be 1% or less. This threshold could be confounded by the presence of other sources of inoculum during crop production or by extremely wet production conditions leading to abnormal disease spread. However, the threshold of 1% seed infection provided a useful baseline for determining the efficacy of seed treatments tested in this project.

In agreement with results from project year 1, Hy-TL (current industry standard) provided good control of *B. allii* on onion seed with no deleterious effects on seed germination, although *B. allii* incidence remained higher than 1% in one seed batch. At a rate equivalent to the current SOLA for carrots and parsnip seed, Wakil XL was not sufficiently effective against *B. allii*. When used at a higher dose (10 g product per million seeds), Wakil XL reduced external botrytis levels to 0.3% from 24.8% but was not so effective against internal infection. Seed germination was not affected. As in year 1, the coded formulation consistently reduced both external and internal infection by *B. allii* without affecting seed germination. *B. allii* could not be detected in either seed lot following use of the highest rate (4n) while use of the standard rate (n) reduced the incidence of infection to 1% or less. Further work to determine the effect of this fungicide seed treatment on the subsequent incidence of neck rot in the field would now be warranted.

Table 7. Effect of fungicide treatment on percentage of onion seeds with normal germination.

Fungicide treatment	% onion seed germination			
	Batch 156719	Batch 156720	Batch 156721	Means
1. Untreated control	95.5	90.0	95.0	93.5
2. Hy-TL	96.5	89.0	94.5	93.3
3. Wakil XL Dose A	95.0	88.5	93.5	92.3
4. Wakil XL Dose B	97.5	90.0	97.0	94.8
5. Wakil XL Dose C	94.5	93.0	95.5	94.3
6. Coded formulations Dose A	95.5	92.0	94.0	93.8
7. Coded formulations Dose B	97.5	93.0	95.0	95.2
8. Coded formulations Dose C	96.0	94.0	93.0	94.3
Means	96.0	91.2	94.7	
D.f.				72
S.e.d (treatment) n.s.				1.27
S.e.d (seed batch)***				0.78
S.e.d (treatment. seed batch)n.s.				

156719 – nil botrytis, 156720 – high botrytis, 1596721 – moderate botrytis
n.s. not significant, *** significant at $P<0.001$

Table 8. Effect of fungicide treatment on the incidence of *Botrytis allii* in onion seed

Fungicide treatment	% *Botrytis allii* infection					
	Internal			External		
	Batch 156720	Batch 156721	Means	Batch 156720	Batch 156721	Means
1. Untreated control	5.3	0.3	2.8	24.8	1.8	13.3
2. Hy-TL	2.0	0.0	1.0	1.8	0.0	0.9
3. Wakil XL Dose A	3.3	0.0	1.7	14.3	0.0	7.2
4. Wakil XL Dose B	2.5	0.5	1.5	6.0	0.0	3.0
5. Wakil XL Dose C	3.5	0.0	1.8	0.3	0.3	0.3
6. Coded formulations Dose A	0.5	0.5	0.5	1.0	0.3	0.7
7. Coded formulations Dose B	1.3	0.5	0.9	0.8	0.3	0.6
8. Coded formulations Dose C	0.0	0.0	0.0	0.0	0.0	0.0
Means	2.3	0.2		6.1	0.3	
D.f.			240			240
S.e.d. (treatment)***			1.1			1.4
S.e.d. (seed batch)***			5.6			0.7
S.e.d. (treatment.seed batch)***			1.6			2.0

156720 – high botrytis, 156721 – moderate botrytis
***significant at $P<0.001$

Table 9. Effect of fungicide treatment on the incidence of microbial contaminants on surface sterilised and non-sterilised onion seed from two seed batches

Fungicide treatment	% microbial contaminants					
	Surface sterilised			Non-sterilised		
	Batch 156720	Batch 156721	Means	Batch 156720	Batch 156721	Means
1. Untreated control	2.0	4.3	3.2	23.8	70.3	47.1
2. Hy-TL	11.8	3.8	7.8	2.5	5.8	4.2
3. Wakil XL Dose A	1.5	2.8	2.2	4.8	9.3	7.1
4. Wakil XL Dose B	0.3	4.3	2.3	3.3	6.8	5.1
5. Wakil XL Dose C	1.0	4.5	2.8	1.5	6.8	4.2
6. Coded formulations Dose A	2.0	0.5	1.3	10.3	3.5	6.9
7. Coded formulations Dose B	0.3	2.8	1.6	6.5	5.8	6.2
8. Coded formulations Dose C	0.3	0.5	0.4	2.5	2.0	2.3
Means	2.4	2.9		6.9	13.8	
D.f.			240			240
S.e.d. (treatment)			0.9***			1.4***

S.e.d. (seed batch)	0.4 n.s.	0.7***
S.e.d. (treatment.seed batch)	1.3***	1.9***

156720 – high botrytis, 156721 – moderate botrytis
n.s. not significant, *** significant at $P<0.001$

Table 10. Loading of experimental fungicide formulations on onion seed as determined by HPLC.

Treatment	Dose	Seed batch	Active ingredient code	Loading as % of target
6	N	156719	1	95.23
			2	92.78
		156720	1	94.40
			2	86.60*
		156721	1	89.70*
			2	95.73
7	0.5 n	156719	1	93.20
			2	93.93
		156720	1	84.20*
			2	85.83*
		156721	1	87.87*
			2	95.43
8	4 n	156719	1	93.78
			2	97.53
		156720	1	97.83
			2	98.13
		156721	1	98.60
			2	100.53

*Loadings outside of acceptable range (90-110%)

2.4 Hot water treatment

2.4.1 Objective

To determine the effect of a pre-soak and hot water treatment on percentage infection of onion seeds by *Botrytis allii* and percentage germination of onion seed, with experimental conditions selected based on results from hot water experiments 1 and 2 (Year 1 Annual Report).

2.4.2 Methods

A 10 g composite sample of each seed batch (156719, 156720 and 156721) was treated as shown in Table 11.

Table 11. Pre-soak and hot water treatments used on onion seed batches

	Pre-soak (18 h)	Hot water treatment (°C)	Duration (min)
1	No	-	-
2	Yes	-	-
3	No	45	15
4	No	45	30
5	No	45	45
6	Yes	45	15
7	Yes	45	30
8	Yes	45	45

For the pre-soak treatments, seed samples were soaked in 100 ml sterile distilled water for 18 h at ambient laboratory temperature (20°C). For each temperature treatment, three 1 litre glass beakers, each containing 200 ml distilled water, were placed into a water bath. The water temperature in each beaker was checked to ensure the test temperature was achieved before adding any seeds. When the target water temperature was achieved, a 10 g sample of seed batches 156719, 156720 and 156721 were added to individual glass beakers and agitated gently to ensure all seeds were submerged. The seeds were soaked in hot water for the specified time, after which the water was decanted off through muslin. The seeds were placed on to filter paper to dry in a laminar airflow for 24 hours. Once the seeds were dry they were collected into individual sterile Petri dishes and stored in the fridge at 4°C until seed germination and agar plate tests were set up.

For each treatment, seed germination tests were done on four replicates of 50 seeds (a total of 200 seeds) from each of seed batches 156719, 156720 and 156721. For each temperature treatment, the incidence of *B. allii* was tested for 400 surface sterilised and non-surface sterilised seeds from seed batches 156720 and 156721, on BSM agar.

2.4.3 Results and discussion

The sensitivity of seed to hot water treatments varied significantly with seed batch and presence or absence of a pre-soak (P<0.001) (Table 12). Percentage germination was reduced for batches 156720 and 156721 (high and moderate botrytis) when exposed to hot water treatments (45°C) for 30 min or 45 min, while batch 156719 (nil botrytis) was not affected. However, an 18 h pre-soak enabled hot water treatment (45°C) up to 45 minutes duration to be used safely, irrespective of seed batch.

Table 12. Means tables for the effect of pre-soaking and hot water treatments on the percentage of onion seeds with normal germination in three seed batches

			% onion seed germination			
Pre-soak (18 h)	Temp (°C)	Duration (min)	Batch 156719	Batch 156720	Batch 156721	Means
No	-	-	96.5	92.0	94.5	94.3
No	45	15	97.5	92.5	88.5	92.8
No	45	30	92.5	70.0	76.0	79.5
No	45	45	97.5	81.5	88.0	89.0
Yes	-	-	94.5	93.5	99.0	95.7
Yes	45	15	97.0	93.5	95.5	95.3
Yes	45	30	97.5	97.0	95.0	96.5
Yes	45	45	99.0	93.0	94.0	95.3
Means			96.5	89.1	91.3	
D.f.						72
S.e.d. (pre-soak.duration.seed batch)***						2.7

156719 – nil botrytis, 156720 – high botrytis, 1596721 – moderate botrytis
***Significant at P=0.001

Only low levels of botrytis were detected in seed batch 156721 (1.8% or less). External and internal botrytis were reduced to 1% or less using a hot water treatment (45°C) of 15 min, and were eliminated when treatment durations of 30 or 45 min were used, with or without a pre-soak (Table 13). For seed batch 156720 (high botrytis), all of the hot water treatments reduced external and internal botrytis from 65% or 8%

respectively, to 0.5% or less. All of the hot water treatments reduced but did not eliminate seed contamination due to other micro-organisms (Table 14). Microbial contaminants were mainly *Penicillium* sp., *Mucor* sp. or *Cladosporium* sp.

As in project year 1, the results emphasised that the sensitivity of onion seed to physical treatments, such as hot water treatment, varies considerably with seed lot. Percentage germination was reduced for seed batches infected with botrytis following treatment at 45°C for 30 or 45 min. These seed batches were more susceptible to hot water damage in project year 2, than in project year 1, when a treatment of 45°C for 30 min had no deleterious effect. This observation is in agreement with results from the EU STOVE project on organic vegetable seed (www.stove-project.net), indicating that seed maturity can also effect sensitivity to physical treatments. However, hot water treatment (45°C) for 30 or 45 min preceded by an 18 h soak at ambient temperature, provided effective control of *B. allii* (external and internal) without reducing percentage germination.

Table 13. Effect of hot water treatments on the incidence of *Botrytis allii* on onion seed from two seed batches

Pre-soak (18 h)	Temp (°C)	Duration (min)	% *Botrytis allii* infection			
			Internal		External	
			Batch 156720	Batch 156721	Batch 156720	Batch 156721
No	-	-	8.3	0.5	65.5	1.8
Yes	-	-	6.0	0.5	54.8	0.3
No	45	15	0.0	0.3	0.0	1.0
No	45	30	0.0	0.0	0.0	0.0
No	45	45	0.0	0.0	0.5	0.0
Yes	45	15	0.0	0.0	0.0	0.0
Yes	45	30	0.0	0.0	0.0	0.0
Yes	45	45	0.0	0.0	0.3	0.0

156720 – high botrytis, 1596721 – moderate botrytis

Table 14. Effect of hot water treatments on the incidence of microbial contaminants on surface sterilised and non-sterilised onion seed from two seed batches

Pre-soak (18 h)	Temp (°C)	Duration (min)	% microbial contaminants			
			Surface sterilised		Non-sterilised	
			Batch 156720	Batch 156721	Batch 156720	Batch 156721
No	-	-	1.5	6.3	36.8	62.5
Yes	-	-	9.0	10.3	26.0	68.8
No	45	15	1.8	0.3	46.3	12.8
No	45	30	1.3	0.0	4.0	4.3

No	45	45	0.5	0.3	5.5	16.5
Yes	45	15	1.5	0.8	2.3	5.5
Yes	45	30	1.3	3.5	4.3	2.8
Yes	45	45	3.3	8.0	6.0	3.5

156720 – high botrytis, 1596721 – moderate botrytis

2.5 Disinfectant treatment

2.5.1 Objective

To determine the effect of disinfectant treatments on percentage infection of onion seeds by *Botrytis allii* and percentage germination of onion seed, with experimental conditions selected based on results from a previous disinfectant experiment (Year 1 Annual Report).

2.5.2 Methods

Experiment 1

A 10 g sample of each seed batch (156719, 156720 and 156721) was subjected to each of the disinfectant treatments shown in Table 15.

Table 15. Disinfectant treatments used to treat onion seed against *Botrytis allii* (Experiment 1)

	Disinfectant treatment	Duration
1	Untreated control	-
2	2% Jet 5 (5% w/w peroxyacetic acid)	15 min
3	2% Jet 5 (5% w/w peroxyacetic acid)	60 min
4	2% Jet 5 (5% w/w peroxyacetic acid)	6 h
5	1% sodium hypochlorite	15 min
6	1% sodium hypochlorite	60 min
7	1% sodium hypochlorite	6 h
8	1% Vitafect (quaternary ammonium compound + biguanadine salts)	15 min
9	1% Vitafect (quaternary ammonium compound + biguanadine salts)	60 min
10	1% Vitafect (quaternary ammonium compound + biguanadine salts)	6 h

For each disinfectant treatment, three 1 litre glass beakers, each containing 200 ml of the appropriate disinfectant, were prepared. 10 g seed samples of seed batches 156719, 156720 and 156721 were added to individual glass beakers and agitated gently to ensure all seeds were submerged. The seeds were treated for the specified duration after which time the liquid was decanted off through muslin. The seeds were

rinsed twice for 1 minute in sterile distilled water. The seeds were then placed onto filter paper to dry in a laminar airflow for 24 hours. Once the seeds were dry they were collected into individual sterile Petri dishes and stored in the fridge at 4°C until seed germination and agar plate tests were set up.

For each disinfectant treatment, seed germination tests were done on four lots of 50 seeds (a total of 200 seeds) from each seed batch (Section 2.2.3). For each disinfectant treatment, the incidence of *Botrytis allii* was tested for 400 non-surface sterilised seeds from seed batches 156720 and 156721 on PYLSE agar (Section 2.2.4).

Experiment 2

Based on the results of Experiment 1 and a previous experiment (Year 1 Annual Report), treatments were modified as shown in Table 16, again using 10 g composite samples of seed batches 156719, 156720 and 156721.

Table 16. Disinfectant treatments used to treat onion seed against *Botrytis allii* (Experiment 2)

	Disinfectant treatment	Duration
1	Untreated control	-
2	2% Jet 5	20 min
3	5% Jet 5	20 min
4	10% Jet 5	20 min
5	2% sodium hypochlorite	20 min
6	5% sodium hypochlorite	20 min
7	10% sodium hypochlorite	20 min
8	2% Vitafect	20 min
9	5% Vitafect	20 min
10	10% Vitafect	20 min

For each disinfectant treatment, seed germination tests were done on four lots of 50 seeds (a total of 200 seeds) from each seed batch (Section 2.2.3). For each disinfectant treatment, the incidence of *Botrytis allii* was tested for 400 non-surface sterilised seeds from seed batches 156720 and 156721 on BSM agar (Section 2.2.4).

2.5.3 Results and discussion

Experiment 1

Effects of pre-soak, disinfectant treatments and seed batch are shown in Table 17. There was a significant treatment interaction effect (seed batch.dose.duration) on percentage seed germination (P<0.001) as well as significant main effects. For 2%

Jet 5, there was no effect on seed germination irrespective of soak duration and seed batch. For 1% sodium hypochlorite, a 6 h soak reduced germination for seed batch 156720 (high botrytis) but not the other batches. For 1% Vitafect, germination was reduced for all batches in the 6 h seed soak and for batch 156720 (high botrytis) after 15 min. This result was in contrast to an experiment in year 1 where 1% Vitafect had no effect on germination after 15 min treatment, with greater sensitivity in year 2 due possibly to the increased age of the seed batch.

Treatment with 2% Jet 5 (60 min or 6 h) reduced the incidence of *B. allii* to 0.5% or less (Table 18). Sodium hypochlorite (1%) was only effective against *B. allii* when the soak duration was extended to 6 h. Vitafect (1%) reduced the incidence of *B. allii* to 0.8% or less for all soak durations tested.

Table 17. Means tables for effect of disinfectant treatments on the percentage of onion seeds with normal germination in three seed batches (Experiment 1)

Disinfectant treatment	Duration	% onion seed germination			Means
		Batch 156719	Batch 156720	Batch 156721	
Untreated	-	94.5	83.0	82.0	86.5
2% Jet 5	15 min	94.5	87.4	82.0	88.0
2% Jet 5	60 min	95.0	87.0	79.5	87.2
2% Jet 5	6 h	98.5	84.5	81.0	88.0
1% sodium hypochlorite	15 min	96.0	80.0	88.0	88.0
1% sodium hypochlorite	60 min	90.5	84.0	84.5	86.3
1% sodium hypochlorite	6 h	91.5	74.0	80.0	81.8
1% Vitafect	15 min	91.5	76.0	88.0	85.2
1% Vitafect	60 min	92.5	83.0	87.0	87.5
1% Vitafect	6 h	66.0	74.5	67.5	69.3
Means		91.1	81.3	82.0	
D.f.					90
S.e.d. (seed batch.disinfect. duration)***					3.4

156719 – nil botrytis, 156720 – high botrytis, 1596721 – moderate botrytis
***significant at $P<0.001$

Table 18. Effect of disinfectant treatments on the incidence of *Botrytis allii* in two batches of onion seed (Experiment 1)

Disinfectant treatment	Pre-soak duration	% *Botrytis allii* infection	
		Batch 156720	Batch 156721
Untreated	-	11.0	4.3
2% Jet 5	15 min	1.5	0.5
2% Jet 5	60 min	0.0	0.3

2% Jet 5	6 h	0.5	0.0
1% sodium hypochlorite	15 min	14.0	1.5
1% sodium hypochlorite	60 min	12.3	2.0
1% sodium hypochlorite	6 h	2.0	0.8
1% Vitafect	15 min	0.0	0.5
1% Vitafect	60 min	0.0	0.8
1% Vitafect	6 h	0.3	0.8

156720 – high botrytis, 1596721 – moderate botrytis

Experiment 2

There was a significant interaction effect (seed batch.disinfectant) on percentage onion seed germination (P<0.001) (Table 19). Jet 5 (20 min soak) did not affect seed germination in any of the seed batches, at the doses tested. Sodium hypoclorite reduced germination in seed batches 156719 and 156721, while Vitafect reduced germination in seed batch 156719.

Jet 5 and sodium hypochlorite reduced the incidence of *B. allii* to 0.8% or less, with treatment at 5% (Jet 5) and 10% (both products) for 20 minutes, resulting in nil detection of the fungus (Table 20). The efficacy of Vitafect against *B. allii* was less consistent, although at doses of 5% or 10% for 20 minutes, incidence was reduced to 1% or less. All of the treatments reduced but did not eliminate other microbial contaminants (Table 21).

Of the disinfectants tested, Jet 5 (e.g. 5% for 20 min) gave the most consistent and effective control of *B. allii* in onion seed without affecting onion seed germination.

Table 19. Effect of disinfectant treatments on the percentage of onion seeds with normal germination in three seed batches (Experiment 2)

Disinfectant treatment	Duration	% onion seed germination			
		Batch 156719	Batch 156720	Batch 156721	Means
Untreated control	-	98.0	91.0	95.5	94.8
2% Jet 5	20 min	94.0	95.5	97.5	95.7
5% Jet 5	20 min	98.5	94.0	96.5	96.3
10% Jet 5	20 min	98.0	94.5	98.0	96.8
Means		96.8	94.7	97.3	
2% sodium hypochlorite	20 min	87.0	89.0	86.0	87.3
5% sodium hypochlorite	20 min	73.0	87.5	73.5	78.0

10% sodium hypochlorite	20 min	78.5	87.5	73.5	79.8
Means		79.5	88.0	77.7	
2% Vitafect	20 min	86.5	94.5	95.5	92.2
5% Vitafect	20 min	79.5	95.0	97.0	90.5
10% Vitafect	20 min	81.0	92.0	92.5	88.5
Means		82.3	93.8	95.0	
D.f.					90
S.e.d. (Seed batch. disinfectant)***					2.4

156719 – nil botrytis, 156720 – high botrytis, 1596721 – moderate botrytis
***significant at $P<0.001$

Table 20. Effect of disinfectant treatments on the incidence of *Botrytis allii* in two batches of onion seed (Experiment 2)

Disinfectant	Duration	% *Botrytis allii* infection	
		Batch 156720	Batch 156721
Untreated control	-	33.5	7.3
2% Jet 5	20 min	0.8	0.3
5% Jet 5	20 min	0.0	0.0
10% Jet 5	20 min	0.0	0.0
2% sodium hypochlorite	20 min	0.0	0.8
5% sodium hypochlorite	20 min	0.5	0.0
10% sodium hypochlorite	20 min	0.0	0.0
2% Vitafect	20 min	5.3	0.8
5% Vitafect	20 min	1.0	0.5
10% Vitafect	20 min	1.0	0.5

156720 – high botrytis, 1596721 – moderate botrytis

Table 21. Effect of disinfectant treatments on the incidence of microbial contaminants on onion seed from two seed batches (Experiment 2)

Disinfectant	Duration	% microbial contaminants	
		Batch 156720	Batch 156721
Untreated control	-	23.0	89.3
2% Jet 5	20 min	0.3	2.5
5% Jet 5	20 min	0.3	0.5
10% Jet 5	20 min	0.3	0.3
2% sodium hypochlorite	20 min	1.3	3.5
5% sodium hypochlorite	20 min	0.0	1.3
10% sodium hypochlorite	20 min	0.0	0.8
2% Vitafect	20 min	2.0	4.5
5% Vitafect	20 min	1.8	1.8
10% Vitafect	20 min	2.3	1.5

156720 – high botrytis, 1596721 – moderate botrytis

2.6 Project conclusions

2.6.1 Methods

- Onion seed batches naturally infested with *B. allii* were sourced from a commercial seed company. Three different seed batches from a single cultivar were used in each experiment to ensure that different treatment methods were evaluated against seed with different levels of *B. allii* internal infection (external and internal); nil, moderate and high.
- Based on published literature, seed batches to be used for experimental work in this project were tested for the incidence of *B. allii* by surface sterilising and plating onto selective agar media. Initially Prune Lactose Yeast Agar amended with streptomycin and erythromycin was used. Subsequently, a modified version of Kritzman's agar was used to reduce overgrowth of *B. allii* by other micro-organisms on the seed.

2.6.2 Fungicides

- Two experiments to test the efficacy of fungicide seed treatments against *B. allii* gave promising results. The following were tested: Hy-TL (industry standard), Wakil XL (three doses), Raxil, and three doses of an experimental seed treatment formulation (containing a combination of two active ingredients).
- Raxil significantly reduced percentage seed germination in the first experiment and was not included for further testing.
- Wakil XL (10 g per million seeds) was effective against external botrytis (even for a seed batch with high contamination levels) but was less effective against internal botrytis. Lower doses did not provide consistent pathogen kill. Seed germination was not affected.
- In two experiments, the higher dose of the experimental formulation eliminated external and internal *B. allii* from a seed batch with high infection levels (25% external, 5% internal) with no deleterious effects on seed germination, even when treated seed had been stored for 5 months. The standard dose reduced *B. allii* incidence to 1% or less. Further work to determine the effect of this fungicide seed treatment on the subsequent incidence of neck rot in the field would now be warranted.

- All of the fungicide treatments reduced but did not eliminate seed contamination due to other micro-organisms.

2.6.3 Hot water treatment

- In three hot water treatment experiments, the treatment effects on seed germination varied according to seed batch, with the seed batches containing moderate and high levels of *B. allii* being more sensitive to treatment than the botrytis-free seed batch.
- Hot water treatments (45°C) for 30 or 45 min provided effective control of *B. allii* but effects on seed germination were dependent on seed batch health and maturity.
- The most promising results were obtained when seed was pre-soaked at 20°C for 18 h prior to hot water treatment (45°C) for 30 or 45 min. These treatments reduced *B. allii* infection to 0.5% or less with no effect on percentage germination, irrespective of seed batch.
- Microbial contamination on seeds was reduced following treatments at 45 or 50°C but not eliminated.

2.6.4 Disinfectants

- The disinfectants Jet 5, sodium hypochlorite and Vitafect were tested using a range of concentrations and soak durations, for their efficacy in eliminating *B. allii* from onion seed and their effects on onion seed germination.
- As with hot water treatment, seed sensitivity to disinfectant treatment varied with seed batch health and maturity.
- Vitafect appeared promising in a first experiment but in subsequent experiments, effects on *B. allii* and seed germination were inconsistent.
- At concentrations and soak durations required to provide effective control of *B. allii*, sodium hypochlorite had a deleterious effect on seed germination.
- Jet 5 provided the most consistent control of *B. allii* with no deleterious effect on seed germination after either 2% Jet 5 for 6 h, or 10% Jet 5 for 20 min, irrespective of seed batch. No *B. allii* was detected in either of two seed batches following treatment with 5% or 10% Jet 5 for 20 min.

2.7 Technology transfer

- Discussions between K. Green and agro-chemical company on possible future use of experimental fungicide formulation for onion seed treatment.
- Any other outputs to be finalised with project coordinator and HDC communication manager.

2.8 References

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2.9 Acknowledgements

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APPENDIX 1. AGAR MEDIA USED FOR TESTING ONION SEEDS FOR INCIDENCE OF *BOTRYTIS ALLII*

Half strength lactic acid PDA (LPDA)

Potato dextrose agar	19.5 g
Agar	5.0 g

In 1 L water with 1.4 ml lactic acid added at 50oC after autoclaving to adjust to pH 4.0

Prune Lactose Yeast Agar (PLY) supplemented with streptomycin and erythromycin (PLYSE)

Basal media

Prune juice	100 ml
Yeast extract	1 g
Lactose	5 g
Agar	30 g
Water	900 ml

Adjust medium to pH 6.0 with aqueous solution of NaOH. Autoclave and cool to 70°C before addition of supplement

Supplement

Streptomycin	0.1 g/L media
Erythromycin	0.1 g/L media

Dissolve both in sterile distilled water before adding to the media

Botrytis Selective Medium (BSM) (modified Kritzman's Agar)

Basal media

$NaNO_3$	1.0 g/L
K_2HPO_4	0.9 g/L
$MgSO_4.7H_2O$	0.2 g/L
KCl	0.15 g/L
Glucose	20.0 g/L
Agar	25.0 g/L

Autoclave and cool to 70°C before the addition of other ingredients

Supplement

Terraclor (PCNB)	0.007 g/L
Prochloraz (as Octave)*	0.2 ppm
Difenoconazole (as Plover)*	4.0 ppm
Chloramphenicol	0.025 g/L
$CuSO_4$	1.7 g/L
Tannic acid	5.0 g/L

The pH of the supplemented basal medium was adjusted to 6.0 with NaOH.

*Prochloraz and difenoconazole were used as replacements for maneb, stated in the original recipe (Kritzman & Netzar, 1978).