Contract report for the
Horticultural Development Council

Bulb onions: Evaluation of alternative seed treatments for the control of neck rot (*Botrytis allii*)

FV 263

April 2006

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The results and conclusions in this report are based on a series of experiments conducted over one year. 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. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

I declare that this work was done under my 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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1.1 Headline

Treatment of onion seed with an experimental fungicidal formulation eliminated external and internal *Botrytis allii*, even from a seed batch containing a high level of the pathogen (18% in the untreated control). There was no deleterious effect of the treatment on percentage seed 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; 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 Prune Lactose Yeast Agar amended with streptomycin and erythromycin.

1.3.2 Fungicide treatment

- An experiment to test the efficacy of fungicide seed treatments against *B. allii* gave promising results. The following products were tested: Hy-TL (industry standard), Wakil XL, Raxil, and three doses of an experimental seed treatment formulation (containing a combination of two active ingredients). Raxil reduced percentage seed germination whereas the other fungicide treatments slightly increased percentage seed germination compared with the untreated control. All of the fungicides reduced the percentage incidence of internal *B. allii.* Raxil and the experimental formulation (at the two higher doses) eliminated internal *B. allii,* even from a seed batch with high levels of the pathogen (18% in the untreated control).

- A second fungicide treatment experiment is planned, focussing on the experimental formulations and possibly one further fungicide with activity against botrytis. Discussions are ongoing with the agro-chemical company providing the experimental formulation to determine the future availability of this experimental product for vegetable seed.

1.3.3 Hot water treatment

- Two hot water treatment experiments have been done. In both experiments, the effect of temperature treatments on seed germination varied with 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. In the second experiment, treatments of 45ºC for 45 minutes and 50ºC for 15 minutes had a deleterious effect on seed germination in one and two out of the three batches, respectively. No botrytis was detected after a treatment of 45ºC for 30 minutes and there was no reduction in seed germination under these conditions, irrespective of seed batch. A third experiment will be done to optimise further temperature/duration combinations. Other microbial contamination on seeds was reduced following treatments at 45 or 50ºC but not eliminated.

1.3.4 Disinfectant treatment

- Disinfectants soaks (two rates each of sodium hypochlorite, Vitafect, and Jet 5 for 15 minutes) did not reduce seed germination. Effects on botrytis incidence in seed were inconclusive because of low levels of botrytis in the untreated control, although treatments with Vitafect (0.1% and 1%) appeared most promising. This experiment is being repeated using longer treatment durations.

1.4 Financial benefits

The proposed work will highlight effective seed treatment techniques for onion neck rot that could potentially be scaled-up for commercial use. It is also 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

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.

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. This annual report describes first experiments conducted 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.

2.1 Control of seed-borne *Botrytis allii*

2.1.1 Introduction

There is a wealth of literature available on the biology, epidemiology and crop losses associated with onion neck rot. Aspects of the seed-borne nature of the disease have been reviewed by Maude (1996), who noted that the number of affected seeds that can cause economic loss is 1 in 100.

Onion neck rot is most commonly caused by *Botrytis allii,* but *B. aclada, B. byssoidea and B. squamosa* can also cause neck rot symptoms. The species *B. aclada* now comprises two subgroups (A1 and AII) which can be distinguished based on chromosome number and conidial dimensions. Yohalem *et al*. (2003) proposed that *B. aclada* be reserved for the small-spored subgroup (A1) and *B. allii* for the larger-spored subgroup (AII) of *B. aclada*. In this project, no attempt has been made to differentiate *B. allii* from *B. aclada*. *B. allii* is used throughout the experimental section of the report to represent either species unless stated otherwise.

While *B. allii* is generally considered to survive on the seed surface, the fungus has also been recovered from seeds exposed to a brief surface sterilisation treatment, indicating a degree of penetration of the seed coat tissues (Maude & Presly, 1977a; Metcalfe, 2002). Several studies have demonstrated that the incidence of neck rot in storage can be related directly to the incidence of infected onion seed from which the crop was grown, but that prevailing weather conditions can influence this relationship significantly (e.g. Maude & Presly, 1977a; 1977b). It has also been demonstrated that there is a stronger correlation of internally infected seed than infested (surface-contaminated) seed with seedling infection and neck rot of bulbs by *B. allii* (Tylkowska & Dorna, 2001). The significance of seedborne inoculum in development of neck rot can be difficult to determine because of the latent nature of infection and prevalence of alternative sources of inoculum (such as volunteers and debris) (du Toit & Derie, 2004).

A knowledge review for HDC project FV 237a (Green, 2002) provided detailed information on alternative treatments for the control of seed-borne diseases including sections on fungicides, thermotherapy, microwave treatment, UV radiation, disinfectants, plant extracts and biological control. Published research on seed treatments for control of *B. allii* has mainly been confined to fungicidal efficacy (mainly benzimidazoles, e.g. Maude & Presly, 1977b). Although it is probable that seed companies undertake their own research on relevant seed treatments, little of this work is published. The following is an update of the previous knowledge review, focussing on potential alternative seed treatments of particular relevance to the control of seed-borne *B. allii*.

2.1.2 Fungicides

Following the withdrawal of Benlate fungicide, the standard industry seed treatment for onion neck rot is now Hy-TL (thiabendazole + thiram) which has a specific off-label approval for use on onion seed.

While there is a wide range of fungicides available for control of botrytis, few of these are available as seed treatment formulations. Wakil XL containing cymoxanil, fludioxonil, and

metalaxyl-M is approved as a pea seed dressing and also has an off-label approval for treatment of carrot and parsnip seed. One of the active ingredients fludioxonil is also contained in the fungicide Switch which is due to be registered for use against botrytis and sclerotinia on certain UK horticultural crops (e.g. lettuce and strawberry) in 2006/2007 (J. Ogborn, Syngenta, pers. comm.). Given the availability of Wakil XL for the horticultural sector, there is merit in evaluating this seed treatment for its effects on *B. allii*.

In a comparison of nine fungicides applied as seed treatments, du Toit *et al*. (2004) demonstrated that *B. allii* could not be detected in onion seed following treatments with Thiram 42-S (thiram), Pristine WG (boscalid + pyraclostrobin) and Rovral 4F (iprodione) compared with 11% *B. allii* in non-treated seed. Pristine WG also gave the best inhibition of seed-borne *Aspergillus* species and *Penicillium* species. Seebold & Langston (2005) demonstrated in Georgia, USA that a combination of boscalid + pyraclostrobin applied to onions during the growing season (early and mid-season applications) could significantly reduce the incidence of neck rot due to *B. allii* on onions cold-stored for 5 months.

Raxil (25 g/l tebuconazole) has off-label approval for treatment of onion seeds for the control of onion white rot. As this fungicide already has approval as an onion seed treatment, testing its effects versus botrytis is warranted.

2.1.3 Hot water treatment

Based on results from HDC project FV 237a (Green, 2003), hot water treatment is now being used commercially for treatment of celery seed produced to organic standards for control of *Septoria apiicola* (B. Lincoln, pers. comm.). Although hot water treatment has potential for treatment of *B. allii*, previous work by seed companies (not published) suggests that for onion seed there is a very narrow margin between conditions required for effective pathogen kill and those that have deleterious effects on seed germination (van Bilsen, pers. comm.). A preliminary experiment (S.J. Roberts, pers. comm.) suggested that *B. allii* could be reduced to below detectable levels by treatment at 45ºC for 15 minutes although the recommendation from the work was that the method should be tested on a wider range of seed lots/cultivars, with different infection levels.

2.1.4 Disinfectants

HDC Project FV 237a demonstrated the potential for use of disinfectants such as sodium hypochlorite, peroxyacetic acid and Vitafect for controlling seed-borne fungal infection, for example on celery (Green, 2003). Peroxyacetic acid (as Jet 5) both as a soak and as a vapour, was found to be particularly effective against *S. apiicola*. Metcalf (2002) suggested that sodium hypochlorite could have potential for control of *B. allii* on onion seed. Metcalf & Duncan (1999) showed that *B. allii* incidence could be reduced by treatment in 10% sodium hypochlorite for 20 minutes, and demonstrated that seed treated in this way could be washed, air dried, stored for several months and drilled, without any reduction in viability or emergence. In this project, a range of disinfectants will be tested at different concentrations and soak durations, to determine their effect against seed-borne *B. allii* and onion seed germination.

2.1.5 Biological control

There are several examples of biological control agents being investigated for their effect on *B. allii*. Peach *et al.* (1994) reported control of *B. allii* by seed treatment with bacterial cells of an *Enterobacter* species. Du Toit *et al.* (2004) demonstrated *in vitro* activity of *Bacillus subtilis* (formulated as Serenade WG) against *B. allii,* with complete inhibition of mycelial growth at 10 and 100 ppm. When applied as a seed treatment, Serenade WG significantly reduced the incidence of *B. allii* in seed but only from 11% in the untreated control to 7.5%. Yohalem *et al*. (2004) investigated the use of the antagonistic fungi (*Ulocladium atrum* and *Clonostachys rosea*) to prevent development of *B. allii* on onion leaves. The fungi inhibited sporulation of *B. allii* on necrotic leaf tips but mycelial growth to adjacent living tissue was not prevented. Köhl *et al*. (1997) also demonstrated inhibition of *B. allii* sporulation using *U. atrum*, *Chaetomium globosum*, *Gliocladium catenulatum* and *Aureobasidium pullulans*. Use of beneficial micro-organisms as seed treatments for improved crop establishment and health is being investigated in an ongoing HortLINK project (HL0167LFV).

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 (<10ºC, 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. The lids were replaced and the boxes were incubated at 20ºC (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 10-12 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. 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 agar (PLYSE). 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 PLYSE agar – 25 seeds per plate. For each seed batch and treatment, 300 surface sterilised seeds and 300 non-sterilised seeds were plated out to determine the incidence of internal and external botrytis, respectively. This was increased to 400 seeds per batch in later experiments (hot water treatment, Experiment 2). All seeds were incubated at approximately 20ºC 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 for i) percentage seed germination and, ii) proportion of seeds from which botrytis colonies developed, were analysed statistically using generalised linear models (GLMs) in Genstat. While ANOVA makes the assumption that data is normally distributed, GLMs allow analyses of data which do not follow a normal distribution, or where a transformation needs to be applied before normality can be assumed. Data which are proportions will tend to follow a binomial distribution. One way of analysing this data would be to do a transformation in order to normalise the data and analyse the transformed data using ANOVA. The best transformation for binomial data is the logit function, which GLM processes internally to produce an accumulated analysis of deviance. This can be interpreted in much the same way as an analysis of variance. The PREDICT command in Genstat can then be used to get the estimated means and standard errors for each treatment level back transformed so that they are produced on the original scale.

2.3 Fungicide treatment

2.3.1 Objective

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

*Table 3***.** Fungicide treatments tested against *Botrytis allii* on onion seed

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. Raxil ***	25 g/l tebuconazole	80 ml product per 1000,000 seeds	40 ml
5. 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
6. 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
7. 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
8. Peridiam red	-	10 ml per 1 kg seed	2 ml

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)

*** Based on SOLA2407/04

$ Combination of two experimental formulations each containing a different active ingredient; an inert polymer (peridiam red) was also applied to treatments 5, 6 and 7 at the rate of 10 ml/1 kg seed.

2.3.2 Methods

A 200 g composite sample of each seed batch (156719, 156720 and 156721) was subjected to each of the fungicide treatments shown in Table 3. Fungicides were applied as a fluidised-bed film coating at Warwick HRI, Wellesbourne (Dr A. Jukes, pers. comm.). As the inert polymer (peridiam red) was included in treatments 5-7, this was also tested on its own (as a control) in treatment 8. 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 300 non-surface sterilised and 300 surface sterilised seeds from seed batches 156720 and 156721 on PLYSE agar (Section 2.2.4).

Seeds from treatments 5-7 (coded formulations) were sent to the supplier of the experimental fungicide to check for fungicide loading by HPLC.

2.3.3 Results and discussion

Raxil had a deleterious effect on seed germination with 0.5% normal germination or less and so was excluded from the statistical analysis. This result coincides with seed industry observations that Raxil reduces onion seed germination. The remaining fungicide treatments, including the experimental formulations gave a significant increase in percentage seed germination compared with the untreated control and peridiam red (Tables 4 & 5). This is likely to have occurred due to a reduction in microbial seed contamination resulting from fungicide treatment. There was also a significant effect of seed batch on germination, with Batch 156719 (botrytis-free) giving higher percentage germination levels.

In the non-surface sterilised seed, external levels of *B. allii* recorded were low for all treatments (due probably to the presence of surface microbial contaminants preventing the growth of *B. allii*) (Table 6). Fungicide treatment reduced the incidence of external botrytis (Table 8). Nil botrytis was recorded for seeds treated with Raxil and the experimental formulations, irrespective of seed batch. For surface sterilised seed, the incidence of internal *B. allii* in untreated seeds of batch 156720 was 18%. There was a significant effect due to treatment (Tables 6 and 7), with all fungicide treatments reducing the incidence of internal botrytis. Again nil botrytis was recorded for seeds treated with Raxil and the experimental formulations (at doses n and 4 x n). Table 9 shows the loading of the experimental formulations (treatments 5, 6 and 7) on seed batches as determined by HLPC. The majority of applications were in the target dose range as specified by the manufacturer.

The results emphasise that Hy-TL (current industry standard) provides adequate control of *B. allii* on onion seed with no deleterious effects on seed germination. Raxil provided excellent control of *B. allii* but with a severe reduction in seed germination. Wakil XL reduced the incidence of *B. allii* without reducing seed germination, but was no better than the industry standard at the rates used. The experimental formulations (at doses n and 4 x n) eliminated *B. allii* from onion seed, even in a seed batch with a high percentage of *B. allii* infection, without reducing seed germination. These formulations warrant further testing.

Table 4. 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	95.0	92.5	94.5	94.0
2. Hy-TL	98.0	96.0	99.5	97.8
3. Wakil XL	98.5	96.5	97.0	97.3
4. Raxil	0.0	0.5	0.5	0.3
5. Coded formulations Dose A	98.5	96.5	96.5	97.2
6. Coded formulations Dose B	99.5	98.0	96.0	97.8
7. Coded formulations Dose C	99.0	97.0	97.0	97.7
8. Peridiam red	97.0	91.5	93.0	93.8
Means*	97.9	95.4	96.2	

*Excluding Raxil
156719 – nil botrytis, 156720 – high botrytis, 156721 – moderate botrytis

Table 5. Analysis of deviance for effect of fungicide treatment on percentage of onion seeds with normal germination (excluding Raxil).

Change	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
+ Treatment	6	31.750	5.292	3.32	0.007
+ Seed batch	2	14.660	7.330	4.60	0.014
+ Treatment.Seed batch	12	12.095	1.008	0.63	0.807
Residual	63	100.495	1.595		
Total	83	159.001	1.916		

*Table 6***.** 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	18.3	2.3	1.5	0.0	6.3	3.2
2. Hy-TL	0.3	0.0	0.2	1.0	0.0	0.5
3. Wakil XL	4.3	0.3	2.3	1.0	0.3	0.7
4. Raxil	0.0	0.0	0.0	0.0	0.0	0.0
5. Coded formulations Dose A	0.0	0.0	0.0	0.0	0.0	0.0
6. Coded formulations Dose B	0.0	0.3	0.2	0.0	0.0	0.0
7. Coded formulations Dose C	0.0	0.0	0.0	0.0	0.0	0.0
8. Peridiam red	13.3	0.7	7.0	0.0	3.7	1.8
Means	4.5	0.5		0.2	1.3	

156720 – high botrytis, 156721 – moderate botrytis

Table 7. Analysis of deviance for the effect of fungicide treatment on the incidence of *Botrytis allii* in onion seed (internal).

Change	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
+ Treatment	7	256.6399	36.6628	69.49	<.001
+ Seed batch	1	99.9097	99.9097	189.36	<.001
+ Treatment.Seed batch	7	6.5755	0.9394	1.78	0.094
Residual	176	92.8628	0.5276		
Total	191	455.9879	2.3874		

Table 8. Analysis of deviance for the effect of fungicide treatment on the incidence of *Botrytis allii* in onion seed (external)

Change	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
+ Treatment	7	69.5578	9.9368	28.22	<.001
+ Seed batch	1	18.8886	18.8886	53.64	<.001
+ Treatment.Seed batch	7	28.7564	4.1081	11.67	<.001
Residual	176	61.9806	0.3522		
Total	191	179.1834	0.9381		

Table 9. Loading of experimental fungicide formulations on onion seed as determined by HPLC.

Treatment	Dose	Seed batch	Active ingredient code	Loading as % of target
5	N	156719	1	95.00
			2	93.00
		156720	1	96.50
			2	97.88
		156721	1	*87.25
			2	98.13
6	0.5 n	156719	1	96.00
			2	92.25
		156720	1	*84.00
			2	97.00
		156721	1	91.50
			2	*89.50
7	4 n	156719	1	90.13
			2	98.03
		156720	1	97.44
			2	97.81
		156721	1	95.31
			2	97.28

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

2.4 Hot water treatment

2.4.1 Objective

To determine the effect of hot water treatment on percentage infection of onion seeds by *Botrytis allii* and percentage germination of onion seed

2.4.2 Methods

Experiment 1

A 4 g composite sample of each seed batch (156719, 156720 and 156721) was treated at each of the following test temperatures:

23ºC
45ºC
50ºC
55ºC
60ºC

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 4 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 15 minutes after which time 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 4ºC until seed germination and agar plate tests were set up.

For each temperature 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 (Section 2.2.3). For each temperature treatment, the incidence of *Botrytis allii* was tested for 300 surface sterilised and non-surface sterilised seeds from seed batches 156720 and 156721, on PLYSE agar (Section 2.2.4).

Experiment 2

Based on the results of Experiment 1, the experiment was repeated on 10 g composite samples of seed batches 156719, 156720 and 156721, applying the following treatments:

Hot water treatment (ºC)	Duration (min)
Untreated control	0
23	45
45	15
45	30
45	45
50	15
50	30
50	45

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 *Botrytis allii* was tested for 400 surface sterilised and non-surface sterilised seeds from seed batches 156720 and 156721, on PLYSE agar.

2.4.3 Results and discussion

Experiment 1

Percentage normal seed germination was reduced to <10% for each of the seed batches following a hot water treatment at 60ºC and this treatment was excluded from statistical analysis (Tables 10 and 11). There was a significant effect of hot water treatment, with consistently higher percentage normal germination following treatment at 45ºC (compared with ambient). This is likely to have occurred because the incidence of other fungal contaminants on the seed that could potentially reduce germination (e.g. *Penicillium* sp. and *Mucor* sp.), were noticeably reduced (data not presented) following treatment at or above 45ºC. Percentage normal seed germination was reduced following treatment at 55ºC, particularly in seed lot 156720, showing that seed batches can differ in their susceptibility to hot water damage. These results coincide with those of previous data (S.J. Roberts, pers. comm.) indicating that hot water treatment at 45ºC and 50ºC for 15 min does not have a detrimental effect on seed germination.

The effects of hot water treatment on the incidence of *B. allii* in seed were difficult to interpret with only a low incidence of the fungus detected in the untreated controls, but also a low incidence of the fungus in one batch (0.5%) even after treatment at 60ºC (Table 12).

Experiment 2

As in Experiment 1, percentage normal seed germination varied significantly with seed batch, with reduced germination in the two seed lots containing *B. allii* (156720 and 156721) compared to 156719 (Tables 13 and 14). There was also a treatment effect, with seed germination reduced even after a treatment at 50ºC for 15 min, in contrast to results from Experiment 1 and previous findings. There was a slight interaction effect, confirming as in Experiment 1, that seed batches varied in their sensitivity to hot water treatment, with for example treatments at 50ºC reducing germination to a greater extent in 156721 than in

156719. Germination was not affected by a treatment at 45ºC for 30 min in any of the seed batches.

Internal botrytis was eliminated in batch 156720 by hot water treatments at 45 and 50ºC, irrespective of duration, compared with 15% botrytis in the seed immersed in water at 23ºC. Internal botrytis detected for seed lot 156721 and external botrytis detected for both seed batches was low, probably due to the high percentage of microbial contamination (Table 15). Microbial contamination (due mainly to a *Mucor* sp. and a *Penicillium* sp.) was reduced following treatments at 45 or 50ºC but not eliminated (Table 16).

Table 10. Effect of hot water treatments (Experiment 1) on the percentage of onion seeds with normal germination in three seed batches.

Hot water treatment	% onion seed germination			
	Batch 156719	Batch 156720	Batch 156721	Means
1. 23ºC	93.0	91.5	93.0	92.5
2. 45ºC	97.5	93.0	96.0	95.5
3. 50ºC	97.5	90.5	91.0	93.0
4. 55ºC	95.5	61.0	81.0	79.2
5. 60ºC	3.5	0.0	7.5	3.7
Means*	95.9	84.0	90.3	

*Excluding 60ºC

156719 – nil botrytis, 156720 – high botrytis, 156721 – moderate botrytis

Table 11. Analysis of deviance on effect of hot water treatments (Experiment 1) on percentage of onion seeds with normal germination (excluding 60ºC treatment)

Change	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
+ Temp	3	97.658	32.553	30.10	<.001
+ Seed batch	2	69.309	34.654	32.04	<.001
+ Temp.Seed batch	6	25.643	4.274	3.95	0.004
Residual	36	38.937	1.082		
Total	47	231.547	4.927		

Table 12. Effect of hot water treatments (Experiment 1) on the incidence of *Botrytis allii* on onion seed from two seed batches

Hot water Treatment	% *Botrytis allii* infection			
	Internal		External	
	Batch 156720	Batch 156721	Batch 156720	Batch 156721
1. 23ºC	0.0	2.0	9.7	0.7
2. 45ºC	0.0	0.0	0.0	0.3
3. 50ºC	0.3	0.0	0.0	0.0
4. 55ºC	0.3	6.3	0.0	0.0
5. 60ºC	0.0	0.0	0.3	1.0

156720 – high botrytis, 156721 – moderate botrytis

Table 13. Effect of hot water treatments (Experiment 2) on the percentage of onion seeds with normal germination in three seed batches.

Water temperature (ºC)	Duration (min)	% onion seed germination			Means
		Batch 156719	Batch 156720	Batch 156721	
-	-	98.5	94.5	96.5	96.5
23	45	97.0	93.0	93.5	94.5
45	15	97.0	91.0	94.0	94.0
45	30	94.0	93.0	93.5	93.5
45	45	95.0	90.0	88.0	91.0
50	15	91.0	82.0	66.0	79.7
50	30	65.0	47.0	35.5	49.2
50	45	41.0	24.5	30.0	31.8
Means		84.8	76.9	74.6	

156719 – nil botrytis, 156720 – high botrytis, 156721 – moderate botrytis

Table 14. Analysis of deviance on effect of hot water treatments (Experiment 2) on percentage of onion seeds with normal germination.

Change	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
+ Seed batch	2	56.691	28.345	24.80	<.001
+ Treatment	7	1436.351	205.193	179.55	<.001
+ Seed batch.Treatment	14	28.655	2.047	1.79	0.057
Residual	72	82.282	1.143		
Total	95	1603.979	16.884		

Table 15. Effect of hot water treatments (Experiment 2) on the incidence of *Botrytis allii* on onion seed from two seed batches.

Water temperature (ºC)	Duration (min)	% *Botrytis allii* infection			
		Internal		External	
		Batch 156720	Batch 156721	Batch 156720	Batch 156721
-	-	2.8	0.0	0.0	2.0
23	45	14.8	0.8	0.0	3.8
45	15	0.0	0.0	0.0	0.0
45	30	0.0	0.0	0.0	0.5
45	45	0.0	0.0	0.0	0.0
50	15	0.0	0.0	0.0	0.0
50	30	0.0	0.0	0.0	0.0
50	45	0.0	0.0	0.0	0.0

156720 – high botrytis, 156721 – moderate botrytis

Table 16. Effect of hot water treatments (Experiment 2) on the incidence of microbial contaminants on surface sterilised and non-sterilised onion seed from two seed batches.

		% microbial contaminants			
Water temperature (ºC)	Duration (min)	Surface sterilised Batch 156720	Batch 156721	Non-sterilised Batch 156720	Batch 156721
-	-	54.8	74.0	100.0	100.0
23	45	18.5	31.5	100.0	100.0
45	15	14.5	6.3	100.0	84.0
45	30	13.0	1.5	100.0	84.0
45	45	6.8	1.0	95.0	66.0
50	15	8.0	6.5	54.0	6.0
50	30	0.8	0.5	34.0	13.0
50	45	2.0	0.8	12.0	9.0

156720 – high botrytis, 156721 – moderate botrytis

2.5 Disinfectant treatments

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.

2.5.2 Methods

A 10 g sample of each seed batch (156719, 156720 and 156721) was subjected to each of the disinfectant treatments shown in Table 17.

Table 17. Disinfectant treatments used to treat onion seed against *Botrytis allii.*

	Product	Product rate
1	Distilled water	-
2	Jet 5 (5% w/w peroxyacetic acid)	0.2%
3	Jet 5 (5% w/w peroxyacetic acid)	2.0%
4	Sodium hypochlorite	0.1%
5	Sodium hypochlorite	1.0%
6	Vitafect (quaternary ammonium compounds + biguanadine salts)	0.1%
7	Vitafect (quaternary ammonium compounds + biguanadine salts)	1.0%

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 10 minutes after which time the liquid was decanted off through muslin. The seeds were rinsed in two 1 minute washes of 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 4ºC 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 300 non-surface sterilised seeds from seed batches 156720 and 156721 on PYLSE agar (Section 2.2.4).

2.5.3 Results and discussion

Percentage seed germination was reduced in the batches containing *B. allii* compared with the botrytis-free seed batch (Tables 18 and 19). All of the disinfectant treatments led to increased seed germination compared to the untreated control, due probably to a reduction of microbial contaminants on the seed surface.

None of the disinfectant treatments eliminated *B. allii* from both seed batches, although lowest levels of the fungus were recovered from seed treated with Vitafect (Tables 20 and 21). Further experiments will be done with these products using an extended treatment duration.

Table 18. Effect of disinfectant treatments on the percentage of onion seeds with normal germination in three seed batches.

Disinfectant Treatment	% onion seed germination			
	Batch 156719	Batch 156720	Batch 156721	Means
1. Distilled water	93.5	90.5	93.0	92.3
2. 0.2% Jet 5	97.0	94.5	97.5	96.3
3. 2.0% Jet 5	97.5	96.5	96.5	96.8
4. 0.1% Sodium hypochlorite	99.0	96.0	95.0	96.7
5. 1.0% Sodium hypochlorite	95.5	96.0	94.5	95.3
6. 0.1% Vitafect	98.5	97.0	95.5	97.0
7. 1.0% Vitafect	98.5	90.0	97.0	95.2
Means	97.1	94.4	95.6	

156719 – nil botrytis, 156720 – high botrytis, 156721 – moderate botrytis

Table 19. Accumulated analysis of deviance for effect of disinfectant treatments on the percentage of onion seeds with normal germination in three seed batches.

Change	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
+ Treatment	6	20.986	3.498	2.87	0.016
+ Seed batch	2	12.897	6.449	5.29	0.008
+ Treatment.Seed batch	12	19.116	1.593	1.31	0.238
Residual	63	76.849	1.220		
Total	83	129.848	1.564		

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

Disinfectant Treatment	% *Botrytis allii* infection	
	Batch 156720	Batch 156721
1. Distilled water	0.0	3.3
2. 0.2% Jet 5	4.3	0.0
3. 2.0% Jet 5	1.3	0.3
4. 0.1% Sodium hypochlorite	4.0	0.3
5. 1.0% Sodium hypochlorite	5.3	0.0
6. 0.1% Vitafect	0.0	0.7
7. 1.0% Vitafect	0.3	1.3

156720 – high botrytis, 156721 – moderate botrytis

Table 21. Accumulated analysis of deviance on the effect of disinfectant treatments on the incidence of *Botrytis allii* in onion seed.

Change	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
+ Treatment	6	20.1283	3.3547	3.93	0.001
+ Seed batch	1	12.9177	12.9177	15.15	<.001
+ Treatment.Seed batch	6	59.8795	9.9799	11.70	<.001
Residual	154	131.3444	0.8529		
Total	167	224.2699	1.3429		

2.6 Overall conclusions

- 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* infection; 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 Prune Lactose Yeast Agar amended with streptomycin and erythromycin.

- An experiment to test the efficacy of fungicide seed treatments against *B. allii* gave promising results. The following were tested: Hy-TL (industry standard), Wakil XL, Raxil, and three doses of an experimental seed treatment formulation (containing a combination of two active ingredients). Raxil reduced percentage seed germination whereas the other fungicide treatments slightly increased percentage seed germination compared with the untreated control. All of the fungicides reduced the percentage incidence of internal *B. allii.* Raxil and the experimental formulation (at the two higher doses) eliminated internal *B. allii,* even from a seed batch with high levels of the pathogen (18% in the untreated control).

- A second fungicide treatment experiment is planned, focussing on the experimental formulations and possibly one further fungicide with activity against botrytis. Discussions are ongoing with the company providing the experimental formulation to determine the future availability of this experimental product for vegetable seed.

- Two hot water treatment experiments have been done. In both experiments, the effect of temperature treatments on seed germination varied with 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. In the second experiment, treatments of 45ºC for 45 minutes and 50ºC for 15 minutes had a deleterious effect on seed germination in one and two out of the three batches, respectively. No botrytis was detected after a treatment of 45ºC for 30 minutes and there was no reduction in seed germination under these conditions, irrespective of seed batch. A third experiment will be carried out to further optimise temperature/duration combinations. Microbial contamination on seeds was reduced following treatments at 45 or 50ºC but not eliminated.

- Disinfectants soaks (two rates each of sodium hypochlorite, Vitafect, and Jet 5 for 15 minutes) did not reduce seed germination. Effects on botrytis incidence in seed were inconclusive because of low levels of botrytis in the untreated control, although treatments with Vitafect (0.1% and 1%) appeared most promising. This experiment is being repeated using longer treatment durations.

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2.8 Technology transfer

- Planning meeting with Project Co-ordinator (26.07.04)
- Project meeting with Elsoms seeds (07.09.04)

2.9 Acknowledgments

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3. 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 70ºC 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