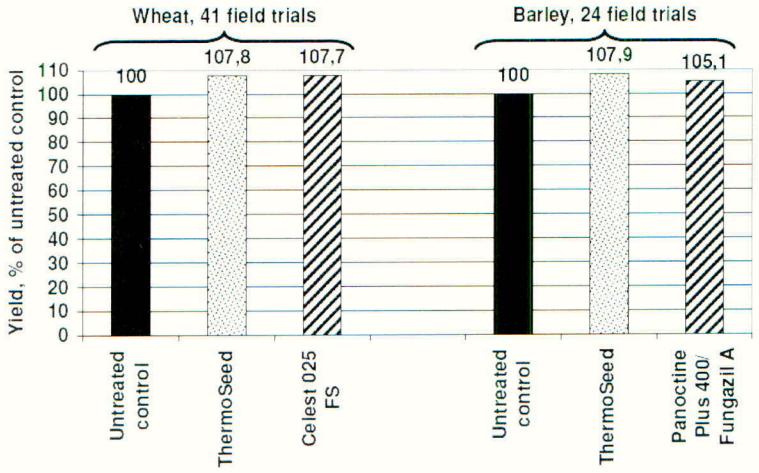

Figure 1 Summary of yield results obtained in field trials at six locations in Sweden during 2003 and 2004, comparing untreated, ThermoSeed-treated and chemically treated seed of wheat and barley. Chemicals used were Celest 025 FS, 2 ml/kg (fludioxinil 25 g/l) in wheat, and for barley either Panoctine Plus 400, 4 ml/kg (imazalil 10 g/l + guazatine 150 g/l) or Fungazil A, 2 ml/kg (imazalil 25 g/l). Data from Johnsson (2003, 2004).

Materials and methods

Ten spinach seed lots (different cultivars) with various pathogen/infestation levels were evaluated. Fourteen carrot seed lots (different cultivars) with various levels of *Alternaria* spp. (*A. dauci*, *A. alternata* and *A. radicina*) were also selected and treated. For each of the crops/lots, untreated controls were compared with the ThermoSeed treatment. In addition, some hot water treatments were also included as a reference in some of the carrot lots used.

The ThermoSeed treatment was performed by exposing the seeds to a hot air/steam mixture for a determined period of time. The treatment recipes are composed of key parameters: time, temperature, air humidity, air flow rate, etc. Due to differences in the heat treatment-related properties among seed lots (Forsberg *et al.*, 2003), the recipes were optimised based on the individual characteristics of each of the seed lots.

Most ThermoSeed treatments were performed using a laboratory treatment device (small scale). Nonetheless, one of the spinach seed lots was treated in a modified large-scale processing unit for cereal seed treatment. The capacity of this machine was reduced from an original 15 t/h to 2 t/h. This large-scale treatment was made after optimisation pre-tests with the laboratory device. The hot water treatments (carrot only) were performed in a similar way/scale to the ThermoSeed laboratory scale treatments.

Germination and plant type testing was performed using both the ISTA paper (blotter) and greenhouse tests. Shelf life was also monitored by storing objects in cardboard tubes in a dark room at 20°C and 40% relative air humidity to compare pre- and post-storage seed quality. Microorganism testing was performed for the following pathogens: carrot, *Alternaria* sp. (*A. dauci*, *A. alternata*, *A. radicina*); spinach, *Stemphylium*, *Verticillium*, *Cladosporium*, *Colletotrichum*, *Fusarium* and *Alternaria*. ISTA standard tests were performed both in-house and by a commercial accredited facility – NAK Tuinbouw Laboratory in the Netherlands.

Results

The results of the disinfection tests are summarised Figure 2 for spinach and in Figure 3 for carrot. The results of the shelf-life tests for both spinach and carrot are summarised in Figure 4.

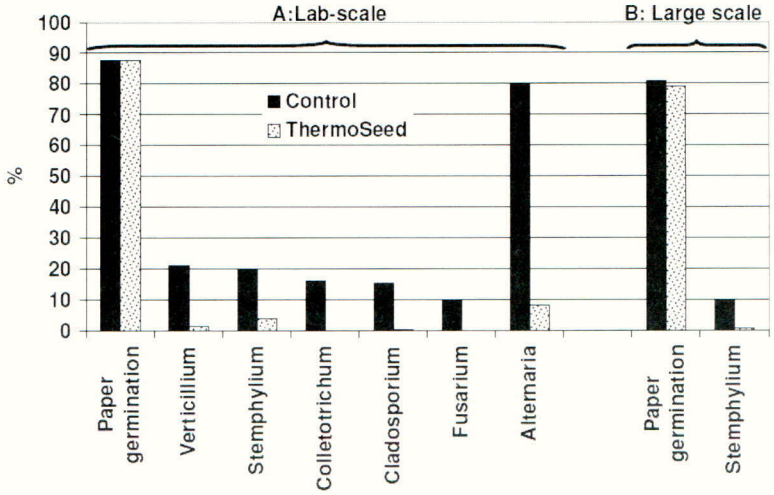

Figure 2 Results of disinfection and germination tests in spinach. (A) Small scale, average of nine seed lots (different origin). (B) Large-scale treatment, one seed lot treated at a 2 t/h capacity in a pilot plant.

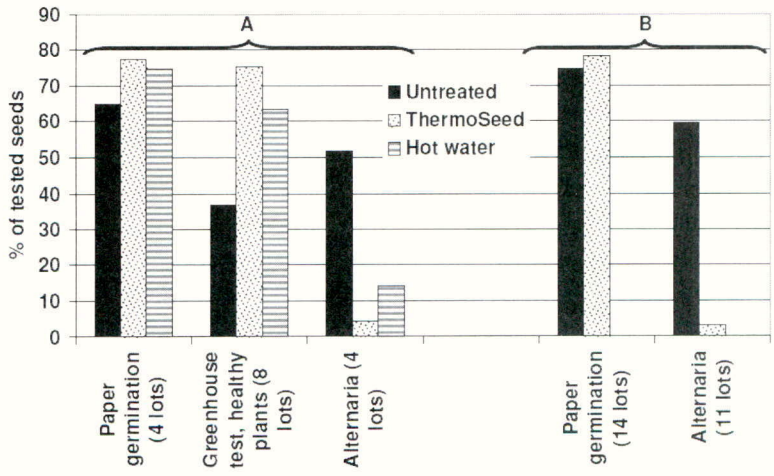

Figure 3 Results of disinfection and germination tests in carrot. (A) Data summarising a number of experiments with four to eight seed lots, where ThermoSeed effects were compared with those of hot water treatment. (B) Data summarising a number of experiments with 11–14 lots, where ThermoSeed effects were tested alone.

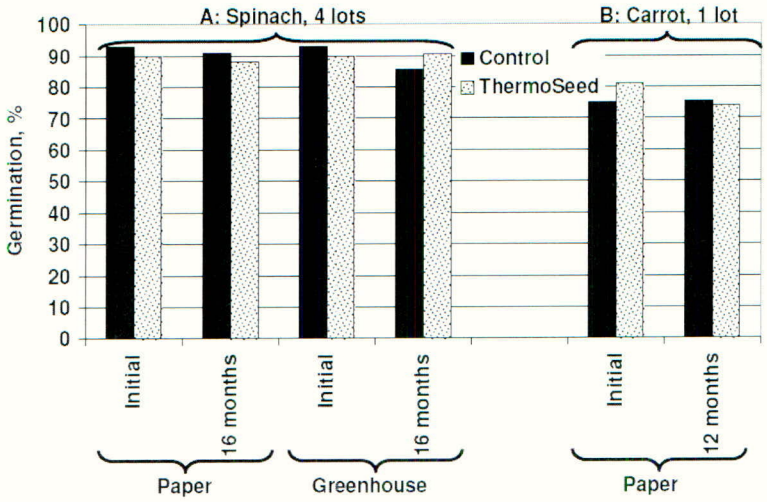

Figure 4 Shelf-life test results. Each test included an untreated control and a ThermoSeed-treated sample. (A) Spinach seed, average of four lots. Germination (paper and greenhouse) evaluated after 16 months. (B) Carrot seed, one lot. Germination on paper evaluated after 12 months.

Discussion

(1) The small-scale treatment tests showed that the ThermoSeed method can:

- efficiently eradicate seed-borne pathogens from nine tested spinach lots
- efficiently eradicate *Alternaria* sp. from highly infected carrot seed lots
- potentially replace hot water treatment for *Alternaria* in carrot
- preserve seed quality in terms of both seed vigour and shelf life.

(2) Although it is so far limited to one seed lot, the large-scale spinach treatment test showed that up-scaling to commercial volumes is feasible with the same kind of machine used for cereals – even though it was not optimally suited for spinach seed.

Similar results were obtained in all seed lots tested, even though they had a different origin and different pathogen spectrum. These tests support previously reported findings from the STOVE project, where other crop/pathogen combinations were also tested with similar results, confirming also good yields. The ThermoSeed method has also shown good potential against *Botrytis*-infected onion seed lots (data not shown). Due to its large volume and economic value, onion is the next crop to be tested. Thus the method has proven to have potential in a wide spectrum of crops and pathogens, and will be developed further.

With that said, it should be remembered that seed lots tend to have very different backgrounds, due to cultural growing practices and environmental conditions. Therefore it is expected to find some seed lots more sensitive than others among treated crops. In order to ensure good vigour and shelf life after treatment, a strict quality control (QC) system has to be set in place. This QC system should individually adjust the treatment to the seed lot characteristics, combining the best disinfection level with optimum effect on quality. In the event that this cannot be achieved (sensitive seed lot), the lot must be rejected before treatment. Currently, a similar

quality system is used for ThermoSeed-treated cereals in Sweden. The system detects the seed lots to be rejected (a small percentage) and optimises the process for the rest. Beyond the ThermoSeed optimisation, the system has proven to be valuable tool in assuring that customers receive seed lots of the highest possible quality.

The up-scaling test was a first pilot trial in which the machine was adjusted to permit the reduced seed flow (from original 15 to 2 t/h) with minimum influence on precision. However, this way of treating the seeds does not permit the same accuracy as when the processing system is designed and dimensioned for the specific crop and capacity from the beginning. Although the spinach results achieved were fully satisfying, a machine designed to handle 2 t/h of spinach seed would be expected to perform even better. For crops such as carrot, onion or other vegetables, even lower capacities would be desired. Currently, this technology is implemented at 1 kg/h, 12 kg/h, 2 t/h and 15 t/h. A suitable adaptation to an intermediate capacity will soon be accomplished. It is foreseen that large machines could be placed in seed companies in large seed-production areas in the near future. Due to the investment costs, we think that small producers will prefer to purchase the treatment in the form of a service under contract, or from specialised seed treaters.

We can see a number of benefits from the developed method.

- ThermoSeed is compatible with any market, and it does not need to be registered in order to be introduced in a new country – since it is not a pesticide.
- Thanks to good effects, high yield and high throughput, the treatment appears to be very competitive in the conventional vegetable production and can add an environmental profile to the various conventional market segments.
- This 'organic' treatment will facilitate organic vegetable production and/or reduce pressure on those chemicals that might be restricted in agriculture in the coming years.
- The method reduces the risk for potential residues in food and environment, and reduces exposure to chemicals of all people involved with the various seed processing stages.
- Treated seed could be combined with either organic or conventional additives, including biological ones.
- ThermoSeed can contribute to goodwill from farmers and their customers to seed producers. Also, farmers can benefit from consumers' goodwill.

Even though we can easily identify the advantages and potential of the ThermoSeed method, there are still challenges to be met. The technology is new for the vegetable market; more work will be needed before we have a commercial-scale machine optimised for vegetable seeds; and more time is needed to develop and evaluate a fully adapted quality system. The potential applications are as many as the different actors. Their approach towards investment in new technologies, strict quality systems and environmental efforts will have to be sorted out for their own niches in order to integrate this kind of technology.

Nonetheless, the trends of public opinion and movements in the industry have been in the favour of this new kind of technology. Companies are increasingly interested in 'green technologies', particularly those that are competitive regardless of their 'green' characteristics. The promising test results achieved in this study with vegetable seed indicates that ThermoSeed has great potential as a vegetable seed treatment too – as already proven for cereals.

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Seed treatment as an additional tool to minimise mycotoxin contamination in cereals

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Fusarium ear blight (FEB), head blight or scab is an important disease of cereals that has reached epidemic stages in many parts of the world in the past decade. The causal organisms of this disease belong to the *Fusarium* species complex. These *Fusarium* pathogens pose a multifaceted threat to yield and quality losses, besides producing significant levels of deleterious mycotoxins for the health of humans and animals. These mycotoxins include trichothecenes and zearalenone. The most prevalent of the trichothecenes are deoxynivalenol (DON) and nivalenol (NIV), for which threshold levels are given by authorities.

As there are no resistant cultivars available, the control of ear blight of wheat relies on an integrated approach comprised of growing a less susceptible cultivar, agronomic practices that reduce the build-up of inoculum, and the use of foliar fungicides applied at anthesis. Furthermore, the levels of these *Fusarium* infections and resulting mycotoxins in cereals can vary from year to year depending on the weather conditions. Research has shown that the effectiveness of foliar fungicides such as triazoles is bound to the timing of the treatment (Matties & Buchenauer, 2000). However, none of the above-mentioned measures allows full control of *Fusarium* infections. With the advent of no-till production practices, the resulting crop residues contribute to increased fungal inoculum carry-over in the straw of previous crops. Examples of this are the *Fusarium* spp. attacking cereals and maize. One of the strengths of the seed treatment compound fludioxonil (trademark Celest or Maxim) is its activity on *Fusarium* spp. and thereby seedling infections, as shown in Table 1. The internal growth of *Fusarium* from lower infection sites within the plant has been reported (Schlüter *et al.*, 2006), but has

Table 1 Activity of fludioxonil against a number of species of *Fusarium* (Ackermann *et al.*, 2007)

Fungal species	**Growth inhibition (EC_{50}, mg a.i./l)**
Fusarium culmorum	0.18
Fusarium graminearum	0.02
Fusarium oxysporum	0.08
Fusarium proliferatum	3.30
Fusarium semitectum	0.01
Fusarium sulphureum	0.09
Monographella nivale	0.15

never been the focus of *Fusarium* studies. Research has focused on the major source of the inoculum, which is known to be airborne spores that infect wheat heads at anthesis.

Syngenta Seed Care initiated novel research on the impact that a fludioxonil seed treatment has on the production of the mycotoxin DON arising from seed infections, crop residues and soil. These studies were conducted in (1) a greenhouse using *F. culmorum*-infested soil; (2) a field microplot trial near Kiel in northern Germany (season 2006/07); (3) a polytunnel semi-field trial utilising *F. culmorum* infested soil (Stein CH, season 2007/08).

Internodes were sampled at different times and analysed by PCR and seeds were harvested and analysed for DNA content and DON. Lastly a phytotron study was conducted to follow the migration of the fungus within the wheat plant by means of GFP transformation and PCR after artificial inoculation of seeds.

In the 2007 greenhouse trial, the fast flowering spring wheat cultivar Apogee was used. The 30 replicates of each treatment were arranged in a complete randomised block design. Inoculation was done by mixing defined amounts of *F. culmorum*-infested organic matter in the soil (Figure 1).

The results of the greenhouse study showed that *Fusarium* infections of wheat can take place at early stages of the plant development via crop residues without any ear infections. A seed treatment with fludioxonil reduced the DON content at harvest by controlling the internal migration of the fungus from the early stages of plant development.

The polytunnel semi-field trial in 2008 was conducted using the highly susceptible winter wheat cultivar Ritmo that was sown into soil containing organic matter heavily infested with *F. culmorum* and *F. graminearum* from artificial inoculations in the two previous years. Ears were covered with bags to avoid ear infections via airborne inoculum. Samples of nodes (1–5), the rachis and kernels were taken at growth stages EC77 and EC93. Ten plants were harvested from each of the four replicates and the samples were pooled. These pooled samples were used for DNA extraction and PCR analysis. The DON content was assayed by means of ELISA (Figure 2).

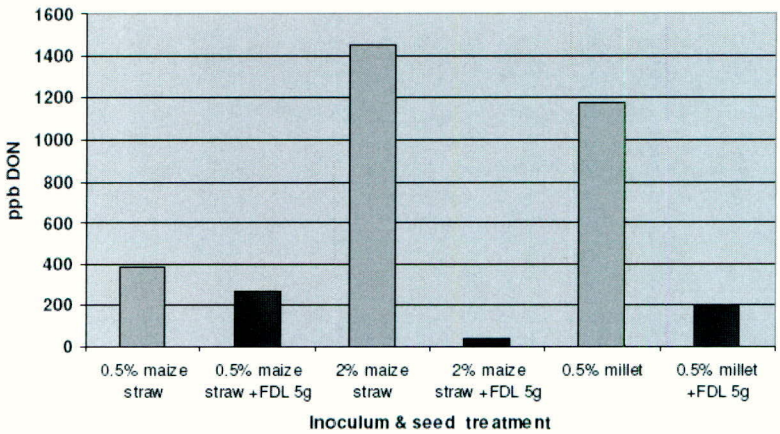

Figure 1 The effect of a fludioxonil seed treatment on the DON content in ears after seedling infections with *Fusarium culmorum* in the greenhouse in 2007

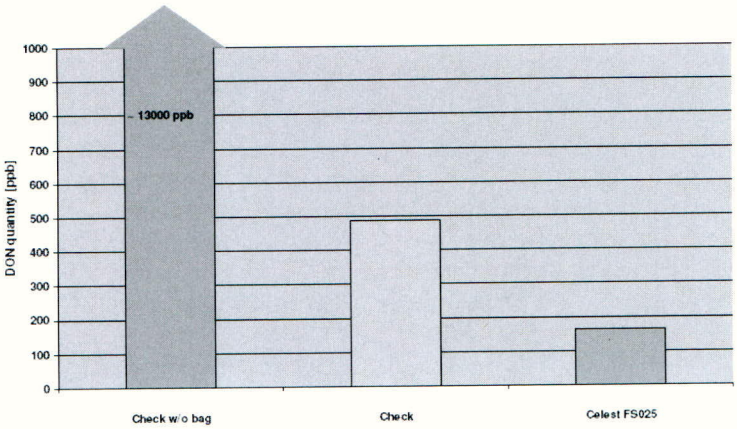

Figure 2 The DON content of kernels after systemic infection with *Fusarium* spp. on wheat cultivar Ritmo at EC93 in polyethylene tunnel semi-field trial (2008)

The *Fusarium* assays showed strong evidence for internal spread via the stem tissues by mainly *F. culmorum*, evidence for *F. graminearum* was low. Therefore it seems that *F. culmorum* has a competitive advantage over *F. graminearum* when infestations take place from infested soil. Results on the DON detections were as follows. (1) At EC77 for all treatments, the content was <300 ppb and no influence of the seed treatment was observable. (2) At EC93, the DON content was ≈500 ppb in the untreated control while the treated seed had a marked reduction in DON to 165 ppb. (3) The importance of bagging the ears was shown by the difference in DON between the bagged ears containing ≈500 ppb, and the exposed ears with DON levels >10,000 ppb.

A field study conducted in the season 2006/07 was established in microplots near Kiel, in a series of infested soils. Internodes were sampled at different growth stages. The results of this study confirmed (1) internal spread of *F. culmorum* infections in wheat from infested soil; and (2) that DON levels in the ears were reduced by the seed treatment on average by 50% over the series of different types of infested soil (Table 2).

Table 2 DON content (ppb) in wheat kernels cv. Ritmo from a field study near Kiel, Germany 2007

	DON (ppb)				
Treatment	**Sterile soil[1]**	**Inoculated soil[2]**	**SNK[3]**	**Tolk[3]**	**Mean**
None	120	800	691	296	596
FDL[4]	nt*	75	813	6	298

[1]Sterile soil was heat-sterilised before the start of the experiment.
[2]Inoculated soil was artificially inoculated with *F. culmorum*.
[3]Soils taken from sites SNK and Tolk are naturally infested with *F. culmorum*.
[4]FDL = Fludioxonil applied at 5 g a.i./100 kg seed.
*nt = not tested.

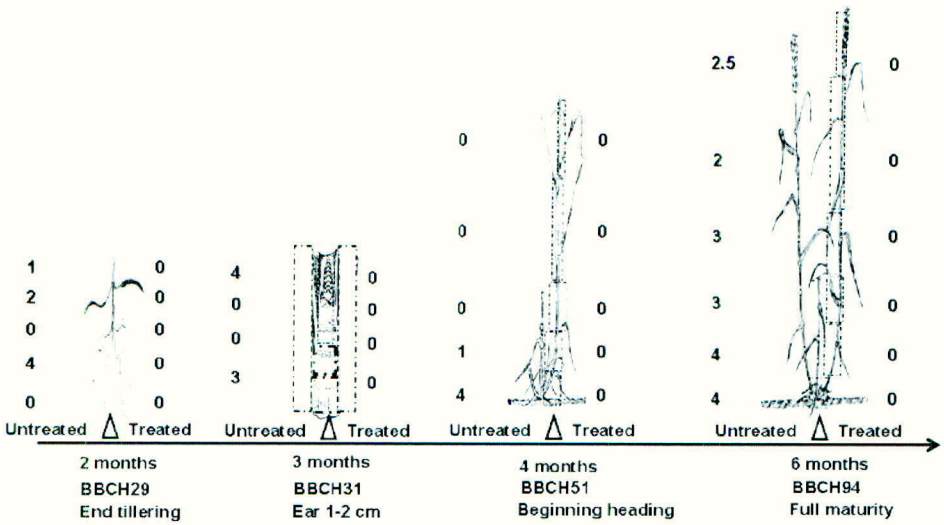

Figure 3 *Fusarium graminearum* progression *in planta* from seed to ear shown by the PCR ratings of a GFP-transformed strain at four growth stages of the wheat plant

A laboratory study was established with researchers at BIOtransfer in France on the internal migration of *F. graminearum* in wheat from the seed to the ear and the impact of a fludioxonil seed treatment on this type of infection. This study utilised a transformed strain of *F. graminearum* containing a green fluorescent protein (GFP). A PCR screen was developed using GFP primers to study the progress of a GFP-transformed strain. The GFP-transformed strain was selected following screening of a number of isolates based on fluorescence, fitness and DON production. The screen was supported by localising the GFP expression *in planta* using fluorescent microscopy. Finally, an assay for DON was conducted on the kernels at maturity. The progression of *F. graminearum* was studied by PCR from seed to ear comparing treated to untreated *F. graminearum*-inoculated seeds. Seeds were sown in pots containing a mixture of compost and vermiculite (75:25) and then placed in a phytotron. A visual rating system was developed for the gene amplification using a scale of 0 = no band to 4 = very strong intensity, and samples were analysed at different growth stages (Figure 3).

The following observations were made. (1) No *Fusarium* was detected by PCR amplification in plants that had received a fludioxonil seed treatment. (2) At GS-94, the assay for DON showed 7650 ppb in the ear of the untreated seed, and no DON was detected in ears from treated seed. (3) This study demonstrates the internal migration of *F. graminearum* from seed to ear and its contribution to final DON expression in the ears.

In summary, infections of wheat at early stages of plant development can contribute to the overall toxin level at harvest. The different studies demonstrate that fludioxonil seed treatments, which show efficacy against *F. graminearum* and *F. culmorum*, offer an additional tool to reduce DON in wheat at harvest, and should be integrated into the approaches taken for reducing *Fusarium* ear blight epidemics on cereals.

Acknowledgements

We would like to thank the institutions and collaborators who contributed to these studies: Klaus Schlüter and Ute Kropf, FH Kiel, Germany and Thierry Barchietto, Anne Le Douarec, Viviane Calaora and Jean-Marc Seng, BIOtransfer, Montreuil, France.

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Seed treatments for the control of onion neck rot (*Botrytis allii*)

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Summary

The efficacy of a range of seed treatments for the control of onion neck rot (*Botrytis allii*) was evaluated to identify potential alternatives to the current industry standard (thiabendazole and thiram) for use in onion production. In a fungicide experiment, nil *B. allii* was detected following use of an experimental seed treatment formulation of boscalid and pyraclostrobin on seed batches with high and moderate botrytis infestation levels. There were no deleterious effects on seed germination. Hot water treatments (45°C) for 30 or 45 min provided effective control of *B. allii*, but effects on seed germination varied with seed batch. When seed was pre-soaked at 20°C for 18 h prior to hot water treatment (45°C) for 30 or 45 min, *B. allii* infestation was reduced to 0.5% or less with no effect on percentage germination, irrespective of seed batch. Of three disinfectants tested, Jet 5 (peroxyacetic acid) provided the most consistent control of *B. allii* with no adverse effect on seed germination after a 20 min soak at concentrations up to 10%, irrespective of seed batch.

Introduction

Infected seed is a major source of inoculum for neck rot (*Botrytis allii*, also known as *B. aclada*), which can lead to significant losses of onions in store if crops are left untreated. The standard industry seed treatment for onion neck rot is Hy-TL (thiabendazole + thiram) which has had a Specific Off-Label Approval (SOLA) for this use since 2002. There is currently retailer pressure to reduce use of thiabendazole, hence concerns within the onion industry that reliance on this single seed treatment for neck rot may be unsustainable. There are also fungicide resistance issues relating to use of MBC fungicides such as thiabendazole (Gladders *et al.*, 1994). This paper describes evaluation of potential alternative seed treatments for onion neck rot control, including fungicides, hot water and disinfectants.

Methods

Seed batches

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 different levels of *B. allii* infestation (internal and/or external); nil (batch A), moderate (batch B) and high (batch C). Seed batches were retrieved from deep-freezer storage and were subsequently stored in controlled environment storage (<10°C, 30% RH) until required for use in laboratory experiments.

Tests to determine the effect of treatments on seed germination were done according to commercial practice, using pleated filter paper enclosed in filter paper wrappers moistened with tap water in clear plastic boxes. Seeds were incubated at 20°C (8 h light/16 h dark) and

percentage 'normal' germination was assessed after 12–14 days. For each treatment, seed germination tests were done for four replicates of 50 seeds from each seed batch.

Incidence and identification of B. allii

The effect of seed treatments on the incidence of *B. allii* and other microbial contaminants was determined by plating seed on Botrytis Selective Medium (BSM) modified from Kritzman's agar (Kritzman & Netzer, 1978) by addition of 4.0 ppm difenoconazole and 0.2 ppm prochloraz as replacement for maneb. Seed samples were immersed in 3% sodium hypochlorite for 1 min then rinsed in two changes of sterile distilled water (SDW) for 1 min each. Seeds were dried on sterile filter paper before plating on agar using sterile technique, with 25 seeds per plate. For each treatment and seed batches B and C, 400 surface-sterilised and 400 non-sterilised seeds were plated to determine the incidence of internal and external botrytis, respectively. All seeds were incubated at approximately 20°C for 5–10 days then examined for the presence of *B. allii.*

Fungicide treatment

A 200 g sample of each seed batch was subjected to the fungicide treatments shown in Table 1. Fungicides were applied as a fluidised-bed film coating at Warwick HRI, Wellesbourne (A. Jukes, pers. comm.). After treatment, seeds were left to air-dry at ambient temperature. Seed germination tests were done as described above. The incidence of *B. allii* and microbial contamination was tested using BSM agar. Statistical analysis was by ANOVA in Genstat.

Table 1 Fungicide treatments tested against *Botrytis allii* on onion seed

Product	Active ingredient	Product dose*	Product dose/200 g†
Untreated control	–	–	–
Hy-TL	225 g/l thiabendazole + 300 g/l thiram	9 ml/kg seed	1.8 ml
Wakil XL Dose 1	50 g/kg fludioxonil + 100 g/kg cymoxanil + 175 g/kg metalaxyl-M	5.0 g/million seeds	0.25 g
Wakil XL Dose 2		7.5 g/million seeds	0.375 g
Wakil XL Dose 3		10.0 g/million seeds	0.5 g
Coded formulations‡ dose 1	Boscalid + pyraclostrobin	*n*	–
Coded formulations‡ dose 2		0.5 × *n*	–
Coded formulations‡ dose 3		4 × *n*	–

*n = standard dose.
†Product dose per 200 g onion seed sample: average of 2500 seeds per 10 g.
‡Combination of two experimental formulations of boscalid and pyraclostrobin (quantities not disclosed) plus an inert polymer (peridiam red).

Hot water treatment

Seeds were hot water-treated at 45°C for 15, 30 or 45 min with or without an 18 h pre-soak in SDW (at 20°C). Control treatments received either nil treatment or an 18 h pre-soak only. Treatment conditions were selected following preliminary experiments testing a range of temperatures (45, 50, 55 and 60°C) and soak durations (Green, 2005). For each treatment, a 10 g sample of each seed batch was placed in 200 ml distilled water at the correct temperature in a water bath. After hot water treatment for the specified duration, the seeds were air-dried in a laminar flow cabinet for 24 h. Seed germination tests were done as described above. The incidence of *B. allii* and microbial contamination was tested by plating on BSM agar.

Disinfectant treatment

Seeds were treated by soaking in 2, 5 or 10% Jet 5 (peroxyacetic acid), sodium hypochlorite or Vitafect containing quaternary ammonium compounds + biguanadine salts (QAC + salts) for 20 min. Treatment conditions were selected following preliminary experiments by Green (2005). After treatment, seeds were rinsed twice for 1 min in SDW then air-dried in a laminar flow cabinet. Seed germination tests were done as described above. The incidence of *B. allii* and microbial contamination was tested using BSM agar.

Results and discussion

Maude & Presly (1977) stated that a neck rot infestation of 10% or more in store can lead to rejection, and that to avoid this situation, 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 infestation provides a useful baseline for determining the efficacy of seed treatments tested.

Fungicide treatments

Percentage normal germination (data not shown) varied significantly with seed batch ($P < 0.001$), with batch B (high botrytis incidence) giving lower percentage germination than the other two batches. There was no significant effect of fungicide treatment on percentage normal germination. All of the fungicide treatments reduced but did not eliminate seed contamination due to other microorganisms. The current industry standard significantly reduced the incidence of *B. allii*, but levels below a 1% threshold were not achieved for Batch B (high botrytis incidence) (Table 2). At a rate equivalent to the current SOLA for carrot and parsnip seed (5 g product per million seeds), Wakil XL was not sufficiently effective against *B. allii*. When used at a higher dose (10 g product per million seeds), the product reduced external botrytis levels to 0.3% from 24.8%, but was not so effective against internal infestation. The experimental formulations of boscalid and pyraclostrobin significantly reduced both external and internal infestation by *B. allii*. Following use of the highest dose ($4 \times n$), *B. allii* could not be detected in either seed lot, while use of the standard dose (n) reduced the incidence to 1% or less. The results on fungicide efficacy were in agreement with findings from an earlier experiment (Green, 2006). Du Toit *et al.* (2004) showed that *B. allii* could not be detected in onion seed following treatments with boscalid + pyraclostrobin (as Pristine WG) compared with 11% *B. allii* in non-treated seed.

Table 2 Effect of fungicide treatment on *Botrytis allii* incidence in onion seed

	Percentage *Botrytis allii* infestation			
	Internal		External	
Fungicide treatment	**Batch B**	**Batch C**	**Batch B**	**Batch C**
Untreated control	5.3	0.3	24.8	1.8
Hy-TL (industry standard)	2.0	0.0	1.8	0.0
Wakil XL Dose 1	3.3	0.0	14.3	0.0
Wakil XL Dose 2	2.5	0.5	6.0	0.0
Wakil XL Dose 3	3.5	0.0	0.3	0.3
Boscalid + pyraclostrobin (*n*)	0.5	0.5	1.0	0.3
Boscalid + pyraclostrobin (0.5 × *n*)	1.3	0.5	0.8	0.3
Boscalid + pyraclostrobin (4 × *n*)	0.0	0.0	0.0	0.0
d.f.	240		240	
SED (treatment.seed batch)***	1.6		2.0	

Batch B = high botrytis; batch C = moderate botrytis.
***Significant at $P < 0.001$.

Hot water treatments

The sensitivity of seed to hot water treatments varied significantly with seed batch and presence of absence of a pre-soak ($P < 0.001$; Table 3). Percentage germination was reduced for batches B and C (high and moderate botrytis) when exposed to hot water treatment (45°C) for 30 or 45 min, while batch A (nil botrytis) was not affected. However, an 18 h pre-soak enabled hot water treatment (45°C up to 45 min duration) to be used safely irrespective of seed batch. The results emphasised that the sensitivity of onion seed to physical treatments, such as hot water treatment, varies considerably with seed lot. In this experiment, seed batches were more susceptible to hot water damage than in an experiment one year previously (Green, 2006), when a treatment of 45°C for 30 min had no deleterious effect, indicating that seed maturity as well as pathogen infestation can affect sensitivity to physical treatments.

Only low levels of botrytis were detected in seed batch C (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 4). For seed batch B (high botrytis), all of the 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 microorganisms (data not shown). Microbial contaminants were mainly *Penicillium*, *Mucor* or *Cladosporium* species.

Table 3 Effect of pre-soaking and hot water treatments on the percentage of onion seeds with normal germination in three seed batches

Pre-soak (18 h)	Temp (°C)	Duration (min)	Percentage onion seed germination			Mean
			Batch A	Batch B	Batch C	
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
SED (pre-soak.duration.seed batch)***						2.7

Batch A = nil botrytis; batch B = high botrytis; batch C = moderate botrytis.
***Significant at $P < 0.001$.

Table 4 Effect of hot water treatments on *Botrytis allii* incidence in two onion seed batches

Pre-soak (18 h)	Temp (°C)	Duration (min)	Percentage *Botrytis allii* infestation			
			Internal		External	
			Batch B	Batch C	Batch B	Batch C
No	–	–	8.3	0.5	65.5	1.8
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	–	–	6.0	0.5	54.8	0.3
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

Batch B = high botrytis; batch C = moderate botrytis.