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Key worker:	Ms A Shepherd, ADAS Arthur Rickwood
Location of project:	ADAS Arthur Rickwood Mepal, Ely Cambs. CB6 2BA
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The results and conclusions in this report are based on an investigation conducted over one year. The conditions under which the experiment was carried out and the results obtained have been reported with detail and accuracy. However because of the biological nature of this 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.

Dr K R Green
Research Scientist
ADAS Arthur Rickwood

Signature……. Date.…….

Report authorised by:

Dr M Heath
Research Leader, Sustainable Crop Management Business Unit
ADAS Boxworth

Signature…….. Date……..

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GROWER SUMMARY

FV 237a

Celery: Evaluation of alternative seed treatments for the control of *Septoria apiicola* (celery leaf spot)

Headlines

- Hot water treatment (48°C, 30 min) without a pre-soak, is the best option available for treatment of organic celery seed to control *Septoria apiicola* (celery leaf spot) at present.

- Under specific conditions, celery seed treatments with hot water, Jet 5 (peroxyacetic acid) and Wakil XL (cymoxanil + metalaxyl-M + fludioxonil) gave significant reductions in the levels of *Septoria apiicola* in celery seed, without affecting seed vigour. However, the standard industry treatment (warm water thiram soak) was the only treatment tested that eliminated septoria.

Background and expected deliverables

Celery leaf spot (*Septoria apiicola*), also known as 'late blight', is the most destructive disease of field grown crops. Initially seen as small brown spots on leaves and stems, the disease can progress rapidly and render the whole crop unmarketable if left unchecked. Seed-borne inoculum is thought to be the major cause of outbreaks of celery late blight. Seed treatment remains an important component of disease management for celery *Septoria*. Thiram is currently used as the standard seed treatment against the fungus, as a warm water soak. This fungicide will not, however, be permitted for crops grown to organic production standards beyond December 2003. Alternatives to thiram seed treatment are also highly relevant to conventional producers, given continued consumer and retailer pressure for rationalisation of fungicide usage.

The aim of the project is to determine the efficacy of a range of seed treatments for celery *Septoria* that could provide alternatives to thiram, for both conventional and organic celery production. Seed treatments are to be assessed according to their effects on, i) incidence of seed-borne inoculum, ii) seed germination, iii) seedling infection, and iv) seed viability after storage.

The expected deliverables from this work are:

- A knowledge review on alternative treatments for the control of seed-borne diseases, particularly in relation to *Septoria* species.
- Information on the efficacy of a range of seed treatments for celery *Septoria*, enabling the industry to make an informed decision on viable alternatives for use in celery production.

Summary of the project and main conclusions

Seed treatments under the following categories were selected for evaluation, based on a knowledge review carried out in 2002. It was intended that some of the selected treatments, if effective, could be appropriate for organic production while others would be more relevant for conventional production.

- hot water treatment
- Disinfectants (Jet 5 and Vitafect)
- UV treatment
- Microwave treatment
- Essential oils (pine, *Eucalyptus citriadorus* and winter savory)
- *Pythium oligandrum* (Polyversum®)
- Fungicides (Wakil XL, Beret Gold and Thiram)

A source of celery seed naturally infected with *S. apiicola* was used in all of the seed treatment evaluations (hybrid TZ9783; harvested 2001/2002). Two batches of this seed were used throughout the project. Batch 1 (lot 37/1007) which had been thiram-treated by the supplier, was used in experiments to determine the effects of treatments on seed germination. Batch 2 (lot 37/1169) was untreated and was used in experiments to determine the effect of seed treatments on septoria levels. In 2002, the percentage of seeds in batch 2 with viable septoria infection was estimated at 10 %.

In year 1, several methods for quantifying viable septoria on celery seeds were evaluated. In particular, a rapid method to determine the effect of seed treatments on percentage germination of septoria spores released from celery seeds, was used routinely throughout the seed treatment experiments.

Conclusions from seed treatment experiments are as follows:

Hot water. Excellent pathogen kill was obtained using a water pre-soak followed by hot water treatment (48ºC, 30 min) but there was also a reduction in seed germination. Hot water treatment (48ºC, 30 min) without a pre-soak, reduced *S. apiicola* spore germination to 3 % (compared with 34 % in the untreated control), without affecting seed germination either immediately after treatment, or 8 months after storage. The same treatment led to a reduction in the percentage of celery seeds with viable septoria infection (seeds releasing septoria spores that subsequently germinated) to 1 % compared with 18 % in the untreated control. Celery leaves inoculated with a suspension from hot-water treated seed did not develop septoria lesions, while 20 % of leaves inoculated with suspension from the untreated control seed developed typical septoria lesions with pycnidia.

Hot water treatment (48ºC, 30 min) is the best option available for treatment of organic celery seed at present, although further discussion within the industry is needed to determine when this treatment will be applied, in relation to pelleting.

Disinfectants. Promising results were obtained with Jet 5 (5 % peroxyacetic acid), both as a soak and also as a vapour treatment, although further studies would be needed to optimise application rates and treatment durations, to ensure that seed germination was not affected. For example, 20 % Jet 5, reduced *S. apiicola* spore germination to 0.1 %, but had a deleterious effect on seed germination after storage (4 months). Particularly promising results were obtained with a Jet 5 vapour treatment, that would not require seed drying after treatment. There may be scope for further development of seed treatment using Jet 5, given that peroxyacetic acid is now approved as a commodity substance that can be used for treatment of surface-borne fungi on potato seed tubers prior to planting.

UV. Treatment with UV-A, UV-B and UV-C had negligible effect on septoria levels or celery seed germination, due largely to UV absorption by seed pigments.

Microwaves. Microwave treatments of 120 sec or more reduced *S. apiicola* spore germination to approximately 10 % but also had deleterious effects on seed vigour. Reports in the literature of successful elimination of seed pathogens using microwaves, suggest that better results may be obtained using modified conditions.

Essential oils and biological control. The essential oils and biological control agent (Polyversum®) used in these studies were ineffective in reducing seed-borne *S. apiicola*.

Fungicides. The fungicide Wakil XL was effective in reducing *S. apiicola* spore germination without affecting seed germination. Wakil XL could potentially be used as an alternative fungicide treatment, with an off-label approval already available for carrot and parsnip, however cheaper alternatives such as thiram and Jet 5 seem equally or more effective. Beret Gold had no effect on *S. apiicola* in these studies, although better results could probably be achieved with higher application rates.

In a comparison of treatments with the industry standard, very promising results were obtained with hot water, Jet 5 (soak and vapour) and Wakil XL (Table A). However, the industry standard (warm water thiram soak) was still the most effective treatment in terms of pathogen kill without affecting seed vigour and was the only treatment that eliminated *S. apiicola* from celery seed. All treatments reduced *S. apiicola* spore germination to less than 4 %. Soaking in 20 % Jet 5 was the only treatment to significantly reduce seed germination after 22 days.

The experiments carried out in this project looked largely at the effect of individual seed treatments on *S. apiicola* and celery seed germination. Further studies to test treatment combinations may be warranted.

Table A. Effect of seed treatments on *S. apiicola* spore germination and celery seed germination

Treatment	% spore germination	% seed germination	
		After 15 days	After 22 days
1. Untreated control	34.5	99.5	100.0
2. Hot water – 48°C for 30 min	3.5	81.5	95.0
3. Disinfectant – 20 % Jet 5 soak, 1 h	0.9	39.0	70.5
4. Disinfectant – 2 % Jet 5 soak, 1 h	1.7	82.5	98.5
5. Disinfectant – 20 % Jet 5 vapour, 24 h	1.8	70.0	93.5
6. Fungicide – Wakil XL seed treatment	0.9	47.5	92.0
7. Standard – Agrichem flowable thiram	0.0	90.5	99.0
Significance	<0.001	<0.001	<0.001
Df	18	18	18
SED	2.637	4.740	3.047

Financial benefits

Outbreaks of leaf spot caused by *Septoria apiicola* are annually observed on organic celery crops, although there has not been widespread crop loss since 1999. Without the option for use of thiram-treated seed or a prophylactic spray regime in organic celery production, it is anticipated that substantially higher levels of loss due to celery leaf spot, could occur in future. This project has shown that hot water treatment could provide a potential alternative to thiram for controlling *S. apiicola* on organic celery seed, and this could mean the difference between a marketable celery crop and total crop loss.

Action points for the celery industry

- When possible, monitor seed crops to ensure that they are completely free from symptoms of celery leaf spot caused by *Septoria apiicola*
- As the health of seed crops cannot be guaranteed, all celery seed should be treated, as a precaution against *S. apiicola*.
- For conventional celery production, a warm water thiram soak remains the most effective treatment for eliminating *S. apiicola* from seed without affecting seed vigour.
- For organic celery production, hot water treatment is the best available option at present using the following conditions:
  - Do not pre-soak the seed, as this seems to make seed more prone to damage by hot water treatment
  - Treat the seed for 30 min at 48°C

- In this project, hot water treatment did not affect seed longevity, although some reports suggest that it may have deleterious effects on older seed. Therefore, it is advisable to treat newly harvested rather than old seed.
- Further discussion within the industry is needed to determine when hot water treatment will be applied, in relation to pelleting.
- Having ensured that disease-free seed is being used, propagators and growers should follow guidelines for minimising the risk of celery leaf spot development during field production, using cultural and chemical controls outlined in HDC Factsheet 06/01 (to be updated in March 2004).
- In this project, Jet 5 either as a soak or a vapour treatment, was highlighted as a possible alternative seed treatment for control of *S. apiicola*. Given that Jet 5 has approval as a commodity substance for use on potato seed tubers, further studies to optimise application rates and treatment durations for the control of *S. apiicola* on celery seed could be warranted.

SCIENCE SECTION

Introduction

Within the vegetable industry, there is increasing interest in alternative seed treatments for the control of seed-borne diseases, both in conventional production, due to retailers' preference for minimal pesticide usage, and in organic systems.

There is particular concern regarding diseases for which seed represents the primary source of inoculum such as celery leaf spot, caused by *Septoria apiicola*. Celery leaf spot, also known as 'late blight', is the most destructive disease of field grown crops. Initially seen as small brown spots on leaves and stems, the disease can progress rapidly and render the whole crop unmarketable if left unchecked.

Industry efforts have been made to improve the health of celery seed produced overseas, while an ongoing project (HDC FV 237) is addressing the optimisation of fungicide timing as a component of IPM. Seed treatment, however, remains an important component of disease management for celery leaf spot, since a seed infection rate as low as 1:7000 can lead to economic loss (Maude, 1996) and resistant cultivars are unlikely to be available in the near future. Thiram is currently used as the standard treatment for control of *S. apiicola* on celery seed, as a warm water soak. Although the derogation of EU regulation 2092/91 (that allows organic growers to use non-organic seed) has been extended indefinitely, thiram seed treatment will not be permitted for organic production beyond December 2003. Alternatives to thiram seed treatment are also highly relevant to conventional producers, given continued consumer and retailer pressure for rationalisation of fungicide usage.

The aim of the project is to determine the efficacy of a range of seed treatments for celery *Septoria* that could provide alternatives to thiram, for both conventional and organic celery production. Seed treatments are to be assessed according to their effects on, i) incidence of seed-borne inoculum, ii) seed germination, iii) seedling infection, and iv) seed viability after storage.

This final report describes a series of experiments carried out in 2002-2003, to assess a range of seed treatments for the control of *Septoria apiicola* on celery seed. Seed treatments under the following categories were selected for evaluation, based on a knowledge review carried out in Project year 1. It was intended that some of the selected treatments, if effective, could be appropriate for organic production while others would be more relevant for conventional production.

- Hot water treatments
- Disinfectants
- UV treatments
- Microwave treatments
- Essential oils
- Biological control using Polyversum®
- Fungicides

A source of celery seed naturally infected with *S. apiicola* was used in all of the seed treatment evaluations (hybrid TZ9783; harvested 2001/2002). Two batches of this seed were used throughout the project. Batch 1 (lot 37/1007) which had been thiram-treated by the

supplier, was used in experiments to determine the effects of treatments on seed germination. Batch 2 (lot 37/1169) was untreated and was used in experiments to determine the effect of seed treatments on septoria levels. In 2002, the percentage of seeds in batch 2 with viable septoria infection was estimated at 10 %, based on observation of release and germination of spores from seeds plated on agar plates in individual water droplets (see Annual Report, December 2002).

In year 1, several methods for quantifying viable septoria on celery seeds were evaluated. In particular, a rapid method to determine the effect of seed treatments on percentage germination of septoria spores released from celery seeds, was used routinely throughout the seed treatment experiments. Additional methods (described for individual experiments) were used to determine the effects of selected treatments on levels of viable septoria in seed.

1. Evaluation of hot water treatment for controlling septoria on celery seed

Introduction

Three experiments on the effects of hot water treatment were carried out. In Experiment 1, conditions for hot water treatments to be evaluated were selected based on literature reviewed in the project interim report (FV 237a, December 2002). Hot water treatments were tested with or without pre-soaking seeds in water. Treatments were tested for their effects on:

- Percentage germination of celery seed
- Percentage germination of septoria spores

Results from Experiment 1 suggested that pre-soaking seeds prior to hot water treatment was effective in reducing septoria levels but could have a deleterious effect on seed germination. Therefore, one promising hot water treatment (with and without a pre-soak) was repeated (Experiment 2), using more recently harvested seed (from 2002/2003 season) to determine whether the effect of pre-soaking on seed germination was less pronounced on newer seed.

Experiment 3 was carried out to determine the effect of the most promising hot water treatment on:

- The germination and vigour of celery seed after 8 months storage
- The percentage of seeds with viable septoria infection.

Materials and methods

Experiment 1
Two seed batches (harvested in 2001/2002 season) were exposed to the same hot water treatments:
Batch 1 – var. TZ9783 (lot 37/1007) Tozers, 'Clean'
Batch 2 – var. TZ9783 (lot 37/1169) Tozers, 'Septoria infected'

Experimental treatments were as follows:

Treatment	16 hour pre-soak	Hot water treatment
1 (control 1)	No	No
2	No	50°C, 25mins
3	No	48°C, 30 min
4 (control 2)	Yes	No
5	Yes	50°C, 25 min
6	Yes	48°C, 30 min

For batch 1 seed, each treatment was applied to a 1 g seed sample and replicated three times.
For batch 2 seed, each treatment was applied to a 2 g seed sample and replicated three times.

Treatments 4-6 were pre-soaked in 150 ml distilled water overnight (20 h) at ambient temperature.

A hot water bath was heated to the correct temperature before immersing the seed samples, tied loosely in muslin, into glass beakers containing 400 ml of water, already in the water bath. The seed samples were gently agitated during the hot water treatment. After hot water

treatment, the seed samples were removed from the muslin and spread out in a thin layer on absorbent paper in an open tray. The seeds were left on the bench top to dry overnight at ambient temperature.

Seed germination
After batch 1 seeds had been treated, seed germination tests were set up, with a sub-sample of 50 seeds from each replicate. Germination tests were initially set up by placing seed on filter paper (three pieces) moistened with sterile distilled water (SDW) in 9 cm-diameter Petri dishes (10 seeds per dish). Dishes were incubated at 20ºC (8h light / 16h dark). After 4 days, however, a different method was adopted to ensure better seed germination; seeds were transferred to 17.5 x 11.5 x 6 cm clear, plastic boxes containing 17.5 x 11.5 x 2 cm, pleated filter paper, moistened with SDW. Three lines of 50 seeds were placed per box. Boxes were incubated at 20ºC (8h light / 16h dark) for 21 days ensuring the filter paper remained moist. Seed germination was assessed after 14 and 21 days using the following categories:

Normal: Cotyledons at least 50% emerged with no damage to terminal bud. Roots > 1cm.

Weak: Roots 0.5 – 1 cm

Abnormal: Roots <0.5 cm

Ungerminated fresh seed: Seeds which remain firm and apparently viable at the end of the test

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.

[Only normal seedlings were included in the germination percentage calculation]

Quantifying viable S. apiicola on seed
After hot water treatment of batch 2 seeds, each 2 g seed sample was immersed in 15 ml distilled water in a conical flask. Flasks were placed onto an orbital shaker for 2 h. For each flask, 1 ml of liquid was pipetted into a universal tube and centrifuged at 2000 rpm for 10 min. The supernatant was discarded and the pellet re-suspended in 500 ul of distilled water. 100 ul of this spore suspension was spread onto each of 3 plates of potato dextrose agar amended with streptomycin (PDA+S). The plates were incubated at 20ºC for 20 h. After this time, the percentage spore germination was determined by microscopic examination of each plate.

Experiment 2
The seed source for the experiment was var. TZ9779 (lot 37/1365, thiram-treated), harvested in 2002/2003. Treatments were applied to 2 g seed samples and replicated three times.

Experimental treatments were as follows:

Treatment	16 hour pre-soak	Hot water treatment
1	No	No
2	No	48ºC, 30 min
3	Yes	No
4	Yes	48ºC, 30 min

Treatments 3 and 4 were pre-soaked in 150 ml distilled water overnight (20 h) at ambient temperature. Hot water treatments, seed germination tests and determination of septoria levels were carried out as described for Experiment 1.

Experiment 3:
Results from Experiment 1 indicated that hot water treatment at 48ºC for 30 min (no pre-soak) was effective in reducing septoria levels without affecting seed germination. Subsequent studies were carried out to study in more detail the effect of these treatment conditions on seed germination and vigour after storage, and septoria levels in seed.

A 2 g sample of hot water-treated batch 1 seeds (48ºC, 30 min) was stored together with a 2 g sample of the same batch that had not been hot water-treated. Each seed sample was enclosed in two paper bags, which in turn was placed into a sandwich box containing sachets of silica gel. The sandwich box and its contents were then sealed using parcel tape. The box was stored in a dark cupboard for 8 months, after which time, seed germination tests were set up as described for Experiment 1.

Hot water-treated seeds (48ºC, 30 min) of batch 2 were placed, four per plate, onto each of 25 plates of PDA+S (100 seeds in total). A droplet of SDW was added to each seed. Each seed was checked within 20 min under the microscope for release of spores of *S. apiicola* and a record was made of the percentage of seeds with spore release. The plates were incubated for 20 h at 20ºC and then re-checked for spore release and spore germination. The same test was set up for batch 2 seeds that had not been hot water-treated.

Spore suspensions of *S. apiicola* from hot water-treated (48ºC, 30 min) and untreated seed of batch 2 were prepared using the centrifuging method described in Experiment 1. Leaves from a healthy celery plant (10 leaves per treatment) were removed, disinfected with 1% sodium hypochlorite for 30 sec and then rinsed in SDW. The leaves were placed in a 45ºC water bath for 15 sec, then after blotting dry were placed individually into Petri dishes containing two pieces of filter paper moistened with SDW. Each leaf was inoculated with a 50 ul droplet of spore suspension. Leaf wetness was maintained for 6 h after inoculation and Petri dishes were incubated on the bench surface at approximately 20ºC. Septoria lesion development was assessed after 14 days.

Results and discussion

Experiment 1
A general observation was that percentage seed germination was lower than would be expected for the untreated control, and this was probably because the methodology for the seed germination test was finalised during this experiment.

Following hot water treatments, the concentration of septoria spores in the suspension that was plated onto agar was approximately 1 x 10^4 spores/ml. This result indicates that although spore release probably occurs during hot water treatment, the process (using reported conditions) does not completely eliminate septoria spores from seeds.

Hot water treatment for 30 min at 48ºC following a pre-soak was the most effective in reducing the levels of viable septoria in seed, resulting in <1 % spore germination compared with 39 % in the untreated control (Table 1). However both treatments that included a pre-soak resulted in a significant reduction in percentage seed germination. Hot water treatment

for 30 min at 48ºC without a pre-soak was considered the most promising treatment, reducing spore germination to <4 % without adversely affecting seed germination.

Table 1. Effect of hot water treatment on spore germination of *S. apiicola* and celery seed germination, using seed from 2001/2002 season

Hot Water Treatment	% spore germination	% seed germination after	
		14 days	20 days
1. No pre-soak, No treatment	39.1	58.7	64.0
2. No pre-soak, 50ºC 25 min	4.0	34.5	56.5
3. No pre-soak, 48ºC 30 min	2.9	8.8	63.8
4. 16h pre-soak, No treatment	10.3	50.7	64.0
5. 16h pre-soak, 50ºC 25 min	1.3	4.0	41.3
6. 16h pre-soak, 48ºC 30 min	0.4	4.7	37.3
Significance	<0.001	<0.001	0.005
Df	9(1)[a]	10	10
SED	3.54	6.66	6.48

[a]for one replicate of treatment 4, accurate observation of spore germination was not possible because of bacterial contamination, and so was considered as a missing value

Experiment 2

Even when more recently harvested seeds were used, hot water treatment preceded by a water soak led to a significant reduction in seed germination compared with the untreated control (Table 2). Hot water treatment without a pre-soak did not give a significant reduction in seed germination.

Although for some forms of seed treatment pre-soaking may be a useful way of 'activating' the pathogen prior to treatment, leading to increased pathogen kill (J. Claxton, pers. comm.), results from Experiments 1 and 2 suggest that the pre-soak may also make the celery seed more sensitive to heat treatment, resulting in reduced vigour.

Table 2. Effect of hot water treatment on germination of celery seed from 2002/2003 season

Hot Water Treatment	% seed germination after	
	14 days	21 days
1. No pre-soak, No treatment	77.3	79.3
2. No pre-soak, 48ºC 30 min	57.3	69.3
3. 16h pre-soak, No treatment	67.3	76.0
4. 16h pre-soak, 48ºC 30 min	37.3	58.0
Significance	0.021	0.058
Df	6	6
SED	9.01	6.35

Experiment 3

After 8 months storage, hot-water treated seed was initially slower to germinate than untreated seed, but after 22 days had exceeded 95 % germination (Table 3). This result supports earlier advice (MAFF, 1963) that hot water treatment does not affect the longevity of seed, so it is preferable to treat the seed in the harvest year even if it is not to be sown until later.

Table 3. Effect of hot water treatment on celery seed germination after 8 months storage

Hot water treatment	% seed germination	
	After 13 days	After 22 days
1. No pre-soak, No treatment	96.0	97.3
2. No pre-soak, 48ºC for 30mins	76.0	97.3
Significance	0.029	1.00
Df	2	2
SED	3.46	2.31

Hot water treatment (48ºC, 30 min) led to a reduction in the percentage of celery seeds with viable septoria infection (seeds releasing septoria spores that subsequently germinated) to 1 % compared with 18 % in the untreated control (Table 4).

Table 4. Effect of hot water treatment on the percentage of celery seeds infected with septoria

Hot water treatment	% seeds releasing septoria spores at 0 h	% seeds releasing septoria spores at 24 h	% seeds releasing septoria spores that germinate
No pre-soak, No treatment	20	20	18
No pre-soak, 48ºC for 30mins	1	1	1

None of the celery leaves inoculated with a suspension from hot-water treated seed developed septoria lesions, while 20 % of leaves (2 out of 10) inoculated with suspension from the untreated control seed developed typical septoria lesions with pycnidia.

It was generally observed that hot water treatment was effective in reducing other fungal and bacterial contamination on the seed surface. For example, hot water-treated seed samples gave less bacterial contamination on the agar plates used to determine septoria spore germination, and less fungal growth on germinating seeds, compared with untreated controls.

The results support previous literature (e.g. Krout, 1921), showing that hot water treatment of celery seeds (48ºC, 30 min) can substantially reduce viable septoria without significantly affecting seed germination. Septoria spore germination was reduced from 39 % to <4 %, while the percentage of seeds with viable infection was reduced from 18 % to 1 %. Maude (1996) suggests that 1 % seed infection would be more than sufficient to initiate a septoria epidemic, however, Bant & Storey (1952) showed that is was possible to obtain clean celery crops using hot water treatment alone to treat severely infected seed, provided care was taken to minimise other infection sources. In our trials it was promising to note that leaves inoculated with spore suspension from hot water-treated seed did not develop septoria lesions compared with 20 % disease incidence in the untreated control.

2. Evaluation of disinfectants for controlling septoria on celery seed

Introduction

Two disinfectants were tested for their effectiveness in eliminating septoria from celery seed. Jet 5 (5 % w/w peroxyacetic acid) is used routinely as a disinfectant in potato and horticultural markets. Research has shown that it can also eliminate some seed-borne diseases. For example, Hopkins & Thompson (2003) reported that low concentrations of peroxyacetic acid (0.16 %) could eliminate bacterial and fungal pathogens from watermelon seed. In the UK, peroxyacetic acid has approval under the Control of Pesticides Regulations (1986) as a commodity substance that can be used to treat seed potatoes pre-planting for the control of surface-borne plant pathogens (one minute tuber dip in a 50:1 dilution of 5 % peroxyacetic acid formulation). Since peroxyacetic acid breaks down to naturally occurring products, it has a low environmental impact and leaves no residues on treated crops.

Vitafect is a soluble concentrate containing biguanidine salts, benzalkonium chloride, quaternary ammonium compounds and wetting agents. It is used mainly for disinfection of irrigation water systems, for clean-up of rockwool, sand beds and capillary matting, and for surface disinfection. It has also been trialled with some success by at least one seed company to reduce levels of seed-borne pathogens on vegetable seed.

Experiments were carried out to determine the effect of two disinfectant treatments (as seed soaks), each at two rates on:

- Percentage germination of celery seed
- Percentage germination of septoria spores

Further studies were carried out to determine the effect of the most promising disinfectant treatment on:

- The germination and vigour of celery seed after 4 months storage
- The percentage of seeds with viable septoria infection.

Materials and methods

Two seed batches (harvested in 2001/2002 season) were exposed to the same disinfectant treatments:
Batch 1 – var. TZ9783 (lot 37/1007) Tozers, ‘Clean’
Batch 2 – var. TZ9783 (lot 37/1169) Tozers, ‘Septoria infected’

Treatments were as follows:

Treatment	Seed soaked for 1h in
1	Untreated control
2	Distilled water
3	1 % solution of Vitafect
4	0.1 % solution of Vitafect
5	20 % solution of Jet 5 (to give 1 % peroxyacetic acid)
6	2 % solution of Jet 5 (to give 0.1 % peroxyacetic acid)

For seed batches 1 and 2, each treatment was applied to a 2 g seed sample and replicated three times.

Seed samples were tied loosely in muslin and immersed into 1-litre glass beakers containing the disinfectant treatment. During treatment (at ambient temperature), the seed samples were gently agitated. After soaking in the disinfectant treatments, the seed samples were removed from the muslin and spread out in a thin layer on absorbent paper in an open tray. The seeds were left on the bench top to dry overnight at ambient temperature.

Seed germination
After batch 1 seeds had been treated, seed germination tests were set up, with a sub-sample of 50 seeds from each replicate sample (three per treatment). Germination tests were run using a 17.5 x 11.5 x 6 cm clear, plastic box containing a 17.5 x 11.5 x 2 cm, pleated filter paper, moistened with SDW. Three lines of 50 seeds were placed per box. Boxes were incubated at 20ºC (8h light / 16h dark) for 21 days ensuring the filter paper remained moist. Seed germination was assessed after 14 and 21 days.

Quantifying viable S. apiicola on seed
After disinfectant treatment, each 2 g seed sample was immersed in 15 ml distilled water in a conical flask. Flasks were placed onto an orbital shaker for 2 h. For each flask, 1 ml of liquid was pipetted into a universal tube and centrifuged at 2000 rpm for 10 min. The supernatant was discarded and the pellet re-suspended in 500 ul of distilled water. 100 ul of this spore suspension were spread onto each of three PDA+S plates. The plates were incubated at 20ºC for 20 h. After this time the percentage spore germination was determined.

Results indicated that treatment with 20 % Jet 5 gave effective pathogen kill without affecting seed germination and this was selected as the most promising treatment for further studies. Therefore, 2 g samples of seed batches 1 and 2 treated with 20 % Jet 5 (as described above) were used to set up the following tests:

Seed germination after storage
A disinfectant treated sample of batch 1 seeds was stored together with an untreated sample of batch 1 seeds. Each seed sample was enclosed in two paper bags, which in turn was placed into a sandwich box containing sachets of silica gel. The sandwich box and its contents were then sealed using parcel tape. The box was stored in a dark cupboard for 4 months. After which time seed germination tests were set up as described previously.

Percentage of seeds with viable septoria infection
Disinfectant-treated batch 2 seeds were placed four per plate onto each of 25 plates of PDA+S (100 seeds in total). A droplet of SDW was added to each seed. Each seed was checked under the microscope for release of spores of *S. apiicola* and a record was made of the percentage of seeds with spore release. The plates were incubated for 20 h at 20ºC and then re-checked for spore release. The same test was set up for batch 2 seeds that had not been treated with disinfectant (untreated control).

Results and discussion

All of the disinfectant treatments significantly reduced *S. apiicola* spore germination, although soaking in 1 % Vitafect was no more effective than soaking seeds in distilled water (Table 5). The soak in 0.1 % Vitafect resulted in nil spore germination but this was not considered to be a reliable result, given the higher levels of spore germination observed following a soak in 1 % Vitafect. Seed treatment with 20 % and 2 % Jet 5 appeared promising, with spore germination reduced from 62 %, to 0.1 % and 3 %, respectively. After 14 days, there was a reduction in percentage seed germination due to 20 % Jet 5 and 2 % Vitafect, but by day 21 there were no treatment effects on seed germination. Based on these results, treatment in 20 % Jet 5 for 1 h was selected for further study.

Table 5. Effect of disinfectants on *S. apiicola* spore germination and celery seed germination

Disinfectant treatment	% spore germination	% seed germination	
		After 14 days	After 21 days
1. Untreated control	62.2	96.7	98.0
2. Distilled water	11.8	99.3	99.3
3. 1 % Vitafect	11.1	87.3	92.7
4. 0.1 % Vitafect	0.0	90.7	91.3
5. 20 % Jet 5	0.1	89.3	92.7
6. 2 % Jet 5	3.1	94.7	97.3
Significance	<0.001	0.041	0.129
Df	10	10	10
SED	5.52	3.46	3.19

When seeds treated with 20 % Jet 5 were stored for 4 months after treatment, there was a reduction in vigour compared with the untreated control (Table 6). This result suggests that if this treatment is to be used in the future, treated seed should be used immediately rather than putting in storage.

Table 6. Effect of disinfectant treatment on celery seed germination after 4 months storage

Disinfectant treatment	% seed germination	
	After 13 days	After 21 days
1. Untreated	92.0	94.7
2. 20 % Jet 5	15.7	64.7
Significance	0.001	0.001
Df	2	2
SED	2.60	1.16

Observation of spore release from individual seeds showed that zero seeds treated with 20 % Jet 5 released septoria spores even after 24 h, compared to 22 % of seeds in the untreated control.

Results for seed treatment with Jet 5 appear promising, but treatment conditions (e.g. concentration, soak duration) will need to be modified to ensure optimum pathogen kill without compromising seed longevity.

3. Evaluation of UV for controlling septoria on celery seed

Introduction

UV radiation is that part of the electromagnetic spectrum between 200 and 400 nm and is conventionally divided intro three components, UV-A (320-400 nm), UV-B (280-320 nm) and UV-C (200-280 nm). The potential for control of disease on plants and seeds using UV radiation was reviewed in the project interim report (FV 237a, December 2002). For example, Brown *et al.* (2001) showed that UV-C could potentially be used for controlling seed-borne infection due to *Xanthomonas campestris* in brassicas. Based on previous research, high and low doses of UV-A, UV-B and UV-C were assessed for their effects on levels of viable *S. apiicola* on seed and celery seed germination. UV seed treatments were carried out at the University of Lancaster, with subsequent assessments of treatment effects undertaken at ADAS Arthur Rickwood.

Methods

Two seed batches were exposed to the same UV-light treatments:
Batch 1 – var. TZ9783 (lot 37/1007) Tozers, 'Clean'
Batch 2 – var. TZ9783 (lot 37/1169) Tozers, 'Septoria infected'

The methods used to irradiate seed have been widely used at the University of Lancaster to irradiate micro-organisms and, more recently, human cells in culture (Gunasekera *et al*., 1997; Rasanayagam *et al*., 1995; Paul, 2000, Moody *et al.,* 1999). All treatments were accurately quantified using a double monochromator spectroradiometer (Macam SR991). Briefly, irradiation treatments were imposed using either fluorescent lamps providing broad-band UV-B or UV-A (Philips TL40W and TLD36W, respectively), or a monochromatic (254 nm) germicidal lamp for UV-C.

There were six ultra-violet light treatments (UV-A high, UV-A low, UV-B high, UV-B low, UV-C high and UV-C low) and an untreated control. Irradiances (Wm^{-2}) at the surface of the seed samples were adjusted by varying the distance from the lamp to the sample. A range of doses was achieved by varying the duration of exposure, which varied from under one minute to around thirty minutes to achieve the doses required (Table 7). The exact duration was adjusted for each sample to correct for variation between lamps, and so achieve the precise doses required. Before irradiation, seeds were soaked in distilled water for 4 h by covering the seed sample to a depth of 2-3 mm. After irradiation seed samples were blotted dry, and left overnight to dry completely. All manipulations of seed from soaking, irradiation to drying were carried out in a temperature controlled dark room at 22±2ºC.

For seed batches 1 and 2, each treatment was applied to a 2 g seed sample and replicated four times.

Seed germination
After batch 1 seeds had been treated, germination tests were set up, with a sub-sample of 50 seeds from each seed sample. Germination tests were run using a 17.5 x 11.5 x 6 cm clear, plastic box containing a 17.5 x 11.5 x 2 cm, pleated filter paper, moistened with SDW. Three lines of 50 seeds were placed per box. Boxes were incubated at 20ºC (8 h light / 16 h dark) for 21 days ensuring the filter paper remained moist. Seed germination was assessed after 14 days.

Quantifying viable S. apiicola on seed
After UV treatment, each 2 g seed sample was immersed in 15 ml distilled water in a conical flask. Flasks were placed onto an orbital shaker for 2 h. For each flask, 1 ml of liquid was pipetted into a universal tube and centrifuged at 2000 rpm for 10 min. The supernatant was discarded and the pellet re-suspended in 500 ul of distilled water. 100 ul of this spore suspension were spread onto each of 3 PDA+S plates. The plates were incubated at 20ºC for 20 h. After this time the percentage spore germination was determined.

Results and discussion

Although some of the UV treatments (e.g. high UV-B) caused an apparent reduction in septoria spore germination, there was no statistically significant reduction compared with the untreated control (Table 7). There was a reduction in seed germination at 14 days, for the high UV-A and high UV-C treatments, but this effect was slight. The lack of treatment effect on septoria spore germination is surprising, given the success of Brown *et al* (2001) in eliminating a bacterial pathogen from brassica seeds using UV-C radiation at the lower of the doses used in this study. The lack of any clear trend between UV-A, UV-B and UV-C, or between doses indicates that celery seed and/or *Septoria apiicola* are inherently unsuitable for UV treatments.

Table 7. Effect of UV treatment on septoria spore germination and celery seed germination

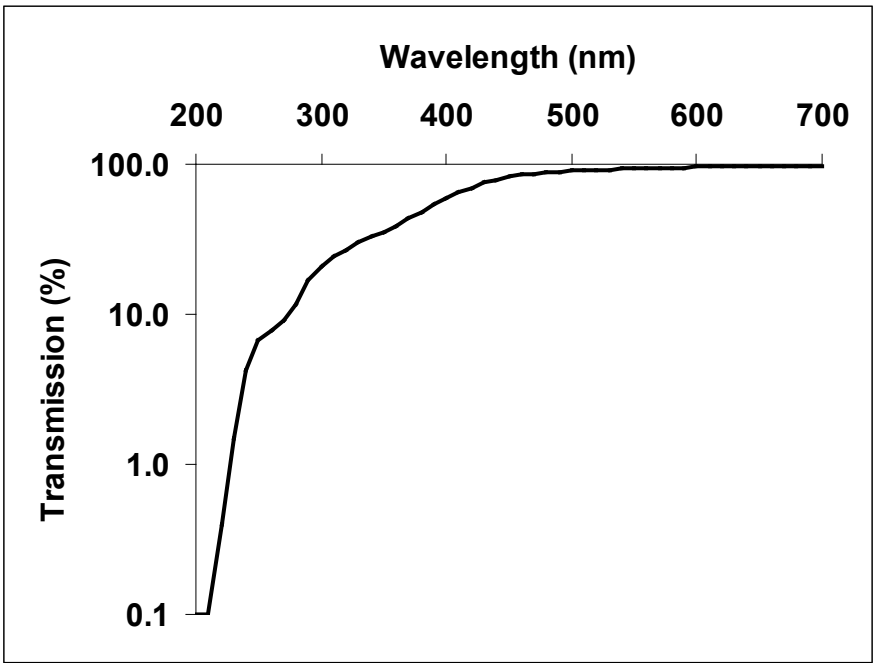
UV-light Treatment	Dose (Wm^{-2})	% spore germination	% seed germination after 14 days
1. Control	0	28.9	94.5
2. High UV-A	105	25.0	82.3
3. High UV-B	105	18.0	89.4
4. High UV-C	105	29.0	82.9
5. Low UV-A	104	22.2	87.9
6. Low UV-B	104	32.7	85.4
7. Low UV-C	104	22.8	92.0
Significance		0.307	0.028
Df		18	18
SED		6.20	3.66

There are a number of reasons that might lead to such insensitivity. Unlike microwave, or hot water treatment, none of the UV treatments that were used here would have penetrated through the whole thickness of the seed. This is a major practical consideration in ensuring that all seeds were uniformly irradiated. The methodology used sought to overcome this issue in two ways. Firstly, to avoid seeds shading each other, the seeds were kept in as near a single layer as possible during irradiation. Secondly, even with a single layer, there are surfaces on the exposed side of the seed, and others on the "shaded" side. Thus, seed were mixed over the course of the irradiation to try to maximise the uniformity of the treatment. Even with mixing, some surfaces may have escaped direct irradiation, and this may account

for some of the variability in the effect of UV treatments on infection. However, the mixing did seem sufficient to minimise the problem, and we do not believe that this was a major contributor to the failure of the UV treatments.

As well as the lack of penetration through the seed, it may be that UV did not effectively penetrate to the target *S. apiicola*, either through the fungal spore case, host tissue, or both. If the pathogen was largely present as spores in surface pycnidia, then treatment would be expected to be effective on all exposed surfaces, assuming that the pathogen is inherently sensitive to UV damage and that treatment penetrates pycnidial walls. By contrast, if infection is more 'deep-seated', then there are additional mechanisms by which tissues might be "protected" via UV screening by plant or fungal tissues. Equally, for UV to induce host defences, it must reach physiologically active tissues at a biologically active dose. The UV absorption properties of tissues in seed or fungal pycnidia are not known, but observations made during irradiations highlighted one potential mechanism for absorption. Seeds were soaked prior to irradiation to initiate the physiological activity in host and pathogen that might be expected to increase induced resistance and damage respectively. However, soaking the seeds produced a solution that appeared dark brown in colour. Such coloration is consistent with soluble phenolics leached from the seed. While such compounds might contribute to UV effects by acting as photosensitisers (e.g. furanocoumarins: Finkelstein *et al.,* 1994; Zobel & Brown, 1993), they also strongly absorb UV, and so might reduce the dose reaching the seeds. Thus, the properties of the solution leached from celery seed were investigated spectrophotometrically. These measurements confirmed that the solution released on soaking was a very effective UV absorber, with transmission declining with decreasing wavelength (Figure 1). Based on these data, the depth of soaking liquid that was present above the seed during treatments would have attenuated the UV dose reaching the seeds by more than 1000-fold for UV-C, approximately 500 fold for UV-B and approximately 250-fold for UV-A. While it is not possible to exclude other mechanisms, which may have further reduced UV penetration, we conclude that the absorption of radiation by the soaking liquid is sufficient to explain the lack of success with UV treatments in this system.

Figure 1. Transmission of UV light at different wavelengths through water used for soaking celery seed



Note: The transmission of the original liquid was too low to provide useful data and the presented data is for a 1:100 dilution of the original liquid at a depth of 10 mm. Data are for means of three replicate samples.

Recommendations.

1. While UV treatments were unsuccessful here, their success in treating brassica seeds shows that this approach can be effective in some systems. On the basis of this study, we recommend that the following preliminary steps should be used as an initial screen to assess the potential of UV for seed treatment and/or the dose range required to be effective.

    1.1. That the inherent UV sensitivity of the pathogen be assessed *in vitro* before seed treatments are attempted. *In vitro* studies are quick and repeatable, and would help quantify the likelihood of successful seed treatments.

    1.2. That the transmission spectrum of the solution produced by soaking seeds should be quantified spectrophotometrically. This would help identify systems, like celery, where compounds leached from the seed greatly attenuate the UV reaching the seed.

2. The steps suggested in 1.1 and 1.2 would provide an initial assessment of the likely dose range required for successful seed treatment. In practice the ability to deliver the required dose will be a function of the UV sources used, and the practical/commercial limits on the length of irradiation. In the current studies the duration of the treatments applied varied between seconds and almost one hour, which was determined by the limits on the intensity of the standard laboratory UV sources that were used. UV sources with far more intense outputs are used in the food industry, and could form the basis of commercial seed treatments of short or very short duration. Such UV sources may have the capacity to deliver effective treatments even for seed infections that require far higher does than were achieved here. The steps described in 1.1 and 1.2 would, therefore, clarify the nature of the UV sources required, both experimentally and commercially.

4. **Evaluation of microwave treatments for control of septoria on celery seed**

Introduction

Examples of microwave treatments being used to eradicate both surface-borne and more deep-seated infection on seeds and vegetative planting material were described and reviewed in the project interim report (HDC FV 237a, December 2002). In this study, selected microwave treatments were tested in two experiments for their effects on celery seed germination and septoria spore germination. Methods for testing spore germination and celery seed germination were under development during Experiment 1, and techniques used for Experiment 2 were slightly modified.

Methods

Two seed batches were exposed to the same UV-light treatments:
Batch 1 – var. TZ9783 (lot 37/1007) Tozers, 'Clean'
Batch 2 – var. TZ9783 (lot 37/1169) Tozers, 'Septoria infected'

Treatments
In Experiment 1, there were five microwave treatments of different durations (30, 45, 60, 90 and 120 sec) and an untreated control (0 sec). Each treatment was applied to a 10 g seed sample and replicated three times.

In Experiment 2, there were six microwave treatments of different durations (60, 90, 120, 150 and 180 sec) and an untreated control (0 sec). Each treatment was applied to a 2 g seed sample and replicated three times.

Microwave treatment
One replicate seed sample (10 g in Experiment 1, 2 g in Experiment 2) was treated at a time. Each seed sample was spread in a single layer on a paper plate. A Hitachi model MR-7350 (1.4 KW; 2450 MHz) microwave was used for the treatments. Treatment duration was timed using a stop-watch rather than relying on the microwave timer.

Seed germination
Experiment 1: After batch 1 seeds had been treated, germination tests were set up, with a sub-sample of 50 seeds from each replicated seed sample. Germination tests were run using 9 cm diameter Petri dishes containing three pieces of filter paper per dish, moistened with SDW, and 10 seeds per dish. Dishes were incubated at 20ºC (8 h light/ 16 h dark) for 21 days, ensuring the filter paper remained moist. Seed germination was assessed after 14 and 21 days.

Experiment 2: After batch 1 seeds had been treated, germination tests were set up, with a sub-sample of 50 seeds from each replicate seed sample. Germination tests were run using a 17.5 x 11. 5 x 6 cm clear, plastic box containing a 17.5 x 11.5 x 2 cm pleated filter paper, moistened with SDW. Three lines of 50 seeds were placed per box. Boxes were incubated at 20ºC (8 h light/16 h dark) for 21 days, ensuring the filter paper remained moist. Seed germination was assessed after 14 and 21 days.

Quantifying viable S. apiicola on seed
Experiment 1: After microwave treatment, seeds from batch 2 were allowed to cool. Once cooled, each 10 g seed sample was immersed in 20 ml distilled water in a conical flask. Flasks

were placed onto an orbital shaker for 5 min. For each flask, the liquid was strained through muslin. The spore suspension was stirred before carrying out four spore counts using a haemocytometer. 100 ul of spore suspension was spread onto each of three plates and incubated at 20ºC for 16-20 h. After this time, the percentage spore germination was determined.

Experiment 2: After microwave treatment, seeds from batch 2 were allowed to cool. Once cooled, each replicate seed sample was immersed in 20 ml distilled water in a conical flask. Flasks were placed on an orbital shaker for 2 h. For each flask, 1 ml of liquid was pipetted into a universal tube and centrifuged at 2000 rpm for 10 min. The supernatant was discarded and the pellet resuspended in 400 ul of distilled water. 100 ul of this spore suspension was spread onto each of three PDA+S plates. The plates were incubated at 20ºC for 16-20 h. After this time, the percentage spore germination was assessed.

Results and discussion

Prior to plating spore suspension onto PDA+S in Experiment 1, the concentration of septoria spores was approximately 3 x 10^5 spores/ml, irrespective of treatment duration. This result indicated that microwave treatments used in this study did not destroy *S. apiicola* spores on or within celery seeds.

There was a significant effect of microwave treatment on septoria spore germination and celery seed germination (Table 8), with increasing treatment duration resulting in decreasing spore and seed germination. However, even after a treatment duration of 120 sec, spore germination still exceeded 16 %.

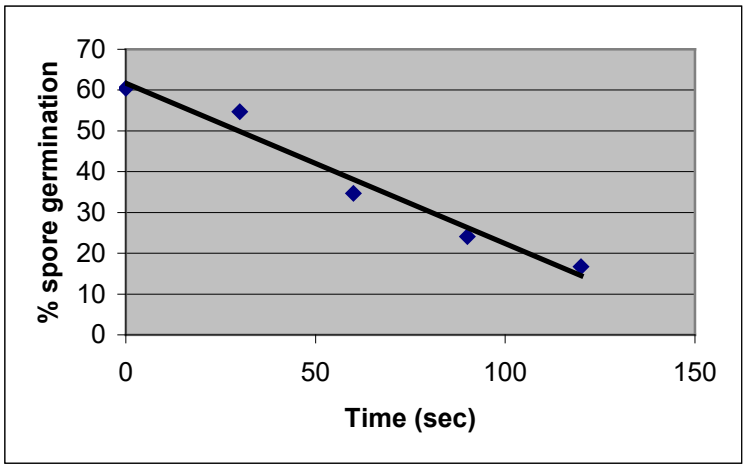
Table 8. Effect of microwave treatment on *S. apiicola* spore germination and celery seed germination (Experiment 1)

Microwave duration (sec)	% spore germination	% seed germination after	
		14 days	21 days
1. 0	60.4	10.7	14.0
2. 30	54.7	8.0	12.6
3. 60	34.7	12.0	18.6
4. 90	24.1	5.3	9.3
5. 120	16.7	0.7	3.3
Significance	0.004		
Df	7(1)[a]		
SED	7.69		

[a]for one replicate of treatment 2, accurate observation of spore germination was not possible because of bacterial contamination, and so was considered as a missing value

There was a strong linear relationship between microwave treatment duration and septoria spore germination (Figure 2), and by extrapolation, this was used to select the lowest duration of microwave treatment that could potentially reduce spore germination to zero, for evaluation in Experiment 2.

Figure 2. Effect of microwave duration on germination of septoria spores from celery seed



$$Y = -0.393X + 61.66$$
$$R^2 = 0.9682$$

In Experiment 2, despite extending the microwave treatment duration to 180 sec, 8 % spore germination was still recorded (Table 9). Use of a more appropriate method for seed germination tests, emphasised the effect of microwave treatment on celery seed germination, with a ten-fold reduction in seed germination after 150 sec microwave duration, compared with the untreated control.

Table 9. Effect of microwave treatment on *S. apiicola* spore germination and celery seed germination (Experiment 2)

Microwave duration (sec)	% spore germination	% seed germination after	
		14 days	21 days
1. 0	28.0	87.3	97.3
2. 60	17.8	61.3	92.0
3. 90	18.2	14.7	75.3
4. 120	8.9	17.3	47.3
5. 150	11.3	2.0	9.3
6. 180	8.0	3.3	47.3
Significance	0.018	<0.001	0.006
Df	10	10	10
SED	4.93	12.73	14.39

Despite the failure of the microwave treatments studied in this project to eliminate celery septoria without affecting seed germination, the range of literature on this subject suggests that there may be scope for achieving better results with modified treatment conditions.

5. Evaluation of essential oils for control of septoria on celery seeds

Introduction

Three essential oils with significant components of compounds previously shown to have anti-microbial activity (Dr R. Cole, pers. comm.) were selected for evaluation, in both the liquid and vapour phase, for activity against septoria:

- Pine oil (containing β-pinene)
- *Eucalyptus citriadorus* oil (containing 1-8 cineol and citral)
- Winter savory oil (containing cavacrol and thymol)

Materials and methods

Two seed batches were exposed to the same treatments:
Batch 1 – var. TZ9783 (lot 37/1007) Tozers, 'Clean'
Batch 2 – var. TZ9783 (lot 37/1169) Tozers, 'Septoria infected'

Treatments were as follows:

Essential oil	Treatment
1. Distilled water	Soak
2. Pine oil (*Pinus sylvestris*)	Soak
3. *Eucalyptus citriadorus*	Soak
4. Winter Savory	Soak
5. Distilled water	Vapour
6. Pine oil	Vapour
7. *Eucalyptus citriadorus*	Vapour
8. Winter Savory	Vapour

For seed batches 1 and 2, each treatment was applied to a 1.5g seed sample and replicated three times.

Essential oil preparation
For each essential oil, 200 ml of a 0.1% solution was prepared, of which 100 ml was decanted into two 250 ml beakers. Each solution was sonicated for 15 min (in a beaker) to ensure the oils were thoroughly dispersed.

Essential oil soak treatment
The hot water bath was heated to 28ºC and the beakers (containing the essential oils) were placed into the water bath. When the oils had reached the correct temperature the seed samples, which were tied loosely in muslin, were immersed in the oil solutions for 1 h. The seed samples were gently agitated during the treatment.

After treatment, the seed samples were removed from the muslin and spread out in a thin layer on absorbent paper in an open tray. The seeds were left on the bench top to dry overnight.

Essential oil vapour phase treatment
For each replicate, one piece of filter paper was placed in the lid of a 9 cm Petri dish. 10 ul of the non-diluted oil or distilled water (control) was pipetted onto the filter paper. As soon as

the droplet was absorbed into the filter paper the 1.5 g seed sample was placed onto the filter paper, in a single layer, and the Petri dish was sealed with parafilm. The Petri dishes were incubated at 28ºC in a CE cabinet for 1 h. After this time the filter papers were removed from the Petri dishes.

Quantifying viable S. apiicola on seed
After essential oil treatment, each 1.5 g seed sample was immersed in 15 ml distilled water in a conical flask. Flasks were placed onto an orbital shaker for 2 h. For each flask, 1 ml of liquid was pipetted into a universal tube and centrifuged at 2000 rpm for 10 min. The supernatant was discarded and the pellet resuspended in 500 ul of distilled water. 100 ul of this spore suspension were spread onto each of three PDA+S plates. The plates were incubated at 20ºC for 20 h. After this time the percentage spore germination was determined.

Results

None of the vapour treatments reduced spore germination in comparison with the untreated control (distilled water, vapour) (Table 10). Although the soak treatments led to a reduction in *S. apiicola* spore germination compared with the vapour treatments, none were significantly better than the distilled water soak. Since the treatments were all ineffective against septoria, we did not proceed with seed germination tests.

Table 10. Effect of essential oil treatments on *S. apiicola* spore germination

Essential oil	Treatment	% spore germination
1. Distilled water	Soak	25.1
2. Pine oil (*Pinus sylvestris*)	Soak	18.7
3. *Eucalyptus citriadorus*	Soak	30.2
4. Winter Savory	Soak	23.2
5. Distilled water	Vapour	48.1
6. Pine oil	Vapour	45.3
7. *Eucalyptus citriadorus*	Vapour	50.9
8. Winter Savory	Vapour	42.1
Significance		<0.001
Df		14
SED		5.88

Within the scope of this project, there was limited opportunity for evaluating essential oils for activity against septoria. While the results in these trials were not promising, it is considered that there may be scope for more effective treatments using other oils and treatment conditions. Given the volatile nature of many essential oils, the use of vapour treatment certainly warrants further investigation, since this would avoid the need for seed drying.

6. Evaluation of a biological control agent for control of septoria on celery seed

Introduction

Pythium oligandrum is a well-documented mycoparasite, and a commercial seed treatment is currently being developed in the UK (Whipps & McQuilken, 2001). A commercial preparation of *P. oligandrum* is already available in the Czech republic as Polyversum® (Biopreparaty). Promotional literature suggests that the seed treatment has activity against a wide range of plant pathogens. The effect of Polyversum® seed treatment on septoria levels and celery seed germination was studied.

Materials and methods

Two seed batches were exposed to the same treatments:
Batch 1 – var. TZ9783 (lot 37/1007) Tozers, 'Clean'
Batch 2 – var. TZ9783 (lot 37/1169) Tozers, 'Septoria infected'

There were two treatments: Treatment one was an untreated control and Treatment 2 was the application of Polyversum®.

For seed batches 1 and 2, each treatment was applied to a 2 g seed sample and replicated three times.

Seed treatment
Each seed sample was placed into a Petri dish and moistened with 2.5 ml SDW. Polyversum® was applied at 0.1 g/2 g seed and mixed thoroughly. After treatment, the seed samples were thoroughly dried by laying the seeds out in a single layer on absorbent paper. The seed samples were allowed to dry out overnight before continuing.

Seed germination
After batch 1 seeds had been treated, germination tests were set up, with a sub-sample of 50 seeds from each seed sample. Germination tests were run using a 17.5 x 11.5 x 6 cm clear, plastic box containing a 17.5 x 11.5 x 2 cm, pleated filter paper, moistened with SDW. Three lines of 50 seeds were placed per box. Boxes were incubated at 20ºC (8h light / 16h dark) for 21 days ensuring the filter paper remained moist. Seed germination was assessed after 14 and 21 days.

Quantifying viable S. apiicola on seed
After treatment, each 2 g seed sample was immersed in 15 ml distilled water in a conical flask. Flasks were placed onto an orbital shaker for 2 h. For each flask, 1 ml of liquid was pipetted into a universal tube and centrifuged at 2000 rpm for 10 min. The supernatant was discarded and the pellet resuspended in 400 ul of distilled water. 100 ul of this spore suspension were spread onto each of 3 PDA+S plates. The plates were incubated at 20ºC for 20 h. After this time the percentage spore germination was determined.

Subsequently, 2 g samples of seed batches 1 and 2 were used to set up the following tests:

1. A Polyversum®-treated sample of batch 1 seeds was stored together with an untreated sample of batch 1 seeds. Each seed sample was enclosed in two paper bags, which in turn was placed into a sandwich box containing sachets of silica gel. The sandwich box and its

contents were then sealed using parcel tape. The box was stored in a dark cupboard for 3 months. After which time seed germination tests were set up as described previously.

2. Polyversum®-treated batch 2 seeds were placed four per plate onto each of 25 plates of PDA+S (100 seeds in total). A droplet of sterile distilled water was added to each seed. Each seed was checked under the microscope for release of spores of *S. apiicola* and a record was made of the percentage of seeds with spore release. The plates were incubated for 20 h at 20ºC and then re-checked for spore release. The same test was set up for batch 2 seeds, which were untreated.

Results and discussion

There was no effect of Polyversum® treatment on either spore germination of *S. apiicola* or celery seed germination immediately after treatment or after 3 months storage (Table 11 and 12). The percentage of seeds releasing spores after 20 h was approximately halved, (from 48 % to 27 %) after Polyversum® treatment.

Initial results with Polyversum® were unpromising, leaving a substantial level of infection in the seed batch. Results from Canada (Lovering *et al.,* 1996) suggest that more effective biological control of *S. apiicola* could be achieved with a *Pseudomonas* species that prevented the formation of pycnidia on agar and inhibited conidial germination on leaf discs.

Use of beneficial micro-organisms (including *P. oligandrum*) for crop establishment and health is being investigated in more detail in an ongoing HortLINK project (HL0167LFV).

Table 11. Effect of Polyversum® on *S. apiicola* spore germination and celery seed germination

Treatment	% spore germination	% seed germination after 14 days
1. No Polyversum®	17.2	89.3
2. Polyversum®	16.9	88.6
Significance	0.729	0.852
Df	2	2
SED	0.84	3.46

Table 12. Effect of Polyversum® on celery seed germination after 3 months storage

Treatment	% seed germination	
	14 days	21 days
1. No Polyversum®	95.3	98.0
2. Polyversum®	92.7	100.0
Significance	0.383	0.225
Df	2	2
SED	2.40	1.16

7. Evaluation of fungicides for the control of septoria on celery seed

Introduction

Beret Gold (fludioxonil) is reported to give moderate control of wheat leaf spot caused by *Septoria tritici*. The active ingredient fludioxonil has been used in other European countries and the USA (marketed as Celest and Maxim) as a seed treatment for sweet corn, sorghum and potatoes. Fludioxonil in combination with cymoxanil and metalaxyl-M (Wakil XL) is used in the UK as a fungicide seed dressing for peas, against *Ascochyta*, damping-off and downy mildew. There is also an off-label approval for use of Wakil XL as a seed treatment for carrots and parsnips in the UK. Given the reported activity of fludioxonil against *Septoria* species and the use of formulations for food crops other than cereals, there is merit in evaluating both Beret Gold and Wakil XL for their effects on *Septoria apiicola* and celery seed vigour.

Materials and methods

Two seed batches were exposed to the same treatments:
Batch 1 – var. TZ9783 (lot 37/1007) Tozers, 'Clean'
Batch 2 – var. TZ9783 (lot 37/1169) Tozers, 'Septoria infected'

There were two fungicide seed treatments, Wakil XL (fludioxonil, cymoxanil and metalaxyl-M) and Beret gold (fludioxonil), and an untreated control.

Fungicides were applied as a fluidised-bed film coating at Horticultural Research International, Wellesbourne, using a fluidised-bed seed-treater (A. Jukes, pers. comm.). Wakil XL was applied at the rate of 5 g product per million seeds (equivalent to 0.3 g Wakil XL per 20 g seed), based on the SOLA for use of Wakil XL on carrot seed. Beret Gold was applied at 400 ul product per 20 g seed, extrapolating from application rates used for cereals. Each treatment was applied to a 2 g seed sample and replicated three times. Seeds were left to air-dry before dispatching to ADAS Arthur Rickwood.

Seed germination
After batch 1 seeds had been treated, germination tests were set up, with a sub-sample of 50 seeds from each seed sample. Germination tests were run using a 17.5 x 11.5 x 6 cm clear, plastic box containing a 17.5 x 11.5 x 2 cm, pleated filter paper, moistened with SDW. Three lines of 50 seeds were placed per box. Boxes were incubated at 20ºC (8 h light/16 h dark) for 21 days ensuring the filter paper remained moist. Seed germination was assessed after 14 and 21 days.

Quantifying viable S. apiicola on seed
After fungicide treatment, seeds from batch 2 were allowed to cool. Once cooled, each 2 g seed sample was immersed in 20 ml distilled water in a conical flask. Flasks were placed onto an orbital shaker for 2 h. For each flask, 1 ml of liquid was pipetted into a universal tube and centrifuged at 2000 rpm for 10 min. The supernatant was discarded and the pellet resuspended in 400 ul of distilled water. 100 ul of this spore suspension was spread onto each of 3 PDA+S plates. The plates were incubated at 20ºC for 16-20h. After this time the percentage spore germination was determined. Where spores were difficult to see, a few drops of lactophenol in cotton blue was added to the plate to enhance the spores' structure and help visual assessment.

2 g samples of seed batches 1 and 2, for all treatments, were used to set up the following tests:

- A fungicide-treated sample of batch 1 seeds was stored together with an untreated sample of batch 1 seeds. Each seed sample was enclosed in two paper bags, which in turn was placed into a sandwich box containing sachets of silica gel. The sandwich box and its contents were then sealed using parcel tape. The box was stored in a dark cupboard for 8 months. After which time seed germination tests were set up as described previously.

- Fungicide-treated batch 2 seeds were placed four per plate onto each of 25 plates of PDA+S (100 seeds in total). A droplet of sterile distilled water was added to each seed. Each seed was checked under the microscope for release of spores of *S. apiicola* and a record was made of the percentage of seeds with spore release. The plates were incubated for 20 at 20ºC and then re-checked for spore release. The same test was set up for batch 2 seeds untreated control.

Results and discussion

Seed treatment with Wakil XL led to a significant reduction in septoria spore germination, without affecting seed germination (Table 13). Spore germination was difficult to observe due to deposit of pigmentation from the fungicide on agar plates but consistent results were obtained in a repeat of the experiment (Table 14). After 3 months storage, there was a delay in germination of Wakil-treated seeds at 15 days, but by 21 days there were no treatment differences (Table 15).

Beret Gold had no effect on septoria spore germination or celery seed germination. There was limited opportunity in the project to trial different fungicide application rates and it is probable that better results could have been achieved with a higher rate.

Table 13. Effect of fungicides on *S. apiicola* spore germination and celery seed germination

Fungicide Treatment	% spore germination	% seed germination after 14 days
1. Untreated control	23.9	97.3
2. Wakil XL	0.3	96.7
3. Beret Gold	22.9	96.7
Significance	0.025	0.871
Df	4	4
SED	5.79	1.44

Table 14. Effect of fungicides on *S. apiicola* spore germination and celery seed germination (repeat)

Fungicide Treatment	% spore germination
1. Untreated control	61.9
2. Wakil XL	1.2
3. Beret Gold	53.2
Significance	<0.001
Df	4
SED	4.99

Table 15. Effect of fungicide treatment on celery seed germination after 3 months storage

Fungicide Treatment	% seed germination	
	After 15 days	After 21 days
1. Untreated control	100.0	100.0
2. Wakil XL	74.6	93.3
3. Beret Gold	96.0	99.3
Significance	0.002	0.116
Df	4	4
SED	3.03	2.64

8. Evaluation of alternative treatments for eliminating Septoria from celery seed, compared with the industry fungicide standard

Introduction

Based on the results from previous studies, the most promising treatments for control of septoria on celery seed were tested in comparison with the industry standard (warm water thiram soak). One treatment that had not been previously tested was also included, because of its potential value if effective (Jet 5 vapour treatment). Treatment effects on percentage germination of celery seed and percentage germination of septoria spores were determined.

Materials and methods

Two seed batches were exposed to the same treatments:
Batch 1 – var. TZ9783 (lot 37/1007) Tozers, 'Clean'
Batch 2 – var. TZ9783 (lot 37/1169) Tozers, 'Septoria infected'

Treatments were as follows:

Treatment	Description
1	Untreated control
2	Hot water – 48ºC for 30 mins
3	Disinfectant – 20 % Jet 5 soak, 1 h
4	Disinfectant – 2 % Jet 5 soak, 1 h
5	Disinfectant – 20 % Jet 5 vapour, 24 h
6	Fungicide – Wakil XL seed treatment
7	Standard – Agrichem flowable thiram (600g l)

For seed batches 1 and 2, each treatment was applied to a 2 g seed sample and replicated four times.

Treatments
Methods for seed treatments 1, 2, 3, 4 and 6 were as described previously.

For treatment 5 (Jet 5 vapour treatment), seed samples were placed in Petri dishes, ensuring that seeds were in a single layer. The Petri dishes were placed in a polythene bag, together with a beaker of 20 % Jet 5. The bag was sealed and left on the laboratory bench at ambient light and temperature. The seeds were agitated after approximately 16 h and were removed after 24 h.

For treatment 7 (thiram), seed batches were soaked for 16 h before soaking for 8 h in thiram (3 ml per litre) at 28ºC. Seeds were left to air dry on absorbent paper.

Seed germination
After batch 1 seeds had been treated, germination tests were set up, with a sub-sample of 50 seeds from each seed sample. Germination tests were run using a 17.5 x 11.5 x 6 cm clear, plastic box containing a 17.5 x 11.5 x 2 cm, pleated filter paper, moistened with SDW. Four lines of 50 seeds were placed per box. Boxes were incubated at 20ºC (8 h light / 16 h dark)

for 21 days ensuring the filter paper remained moist. Seed germination was assessed after 14 and 21 days.

Quantifying viable S. apiicola on seed
After treatment, each 2 g seed sample was immersed in 15 ml distilled water in a conical flask. Flasks were placed onto an orbital shaker for 2 h. For each flask, 1 ml of liquid was pipetted into a universal tube and centrifuged at 2000 rpm for 10 min. The supernatant was discarded and the pellet re-suspended in 500 ul of distilled water. 100 ul of this spore suspension were spread onto each of 3 PDA+S plates. The plates were incubated at 20ºC for 20 h. After this time the percentage spore germination was determined.

For Wakil-treated seed, the spore suspension was centrifuged only for 5 min and the supernatant was plated out directly onto plates of PDA+S.

Results and discussion

All of the treatments reduced septoria spore germination to low levels (less than 4 %) compared with 34 % in the untreated control (Table 16). Thiram was the only treatment to eliminate viable septoria. All of the seed treatments except thiram reduced seed germination at 15 days, but 20 % Jet 5 applied as a soak was the only treatment to reduce seed germination after 22 days.

Table 16. Effect of seed treatments on *S. apiicola* spore germination and celery seed germination

Treatment	% spore germination	% seed germination	
		After 15 days	After 22 days
1. Untreated control	34.5	99.5	100.0
2. Hot water – 48ºC for 30 min	3.5	81.5	95.0
3. Disinfectant – 20 % Jet 5 soak	0.9	39.0	70.5
4. Disinfectant – 2 % Jet 5 soak	1.7	82.5	98.5
5. Disinfectant – 20 % Jet 5 vapour	1.8	70.0	93.5
6. Fungicide – Wakil XL seed treatment	0.9	47.5	92.0
7. Standard – Agrichem flowable thiram	0.0	90.5	99.0
Significance	<0.001	<0.001	<0.001
Df	18	18	18
SED	2.637	4.740	3.047

The results emphasise that while very significant reductions in pathogen levels can be obtained with alternative treatments, thiram still remains the most effective treatment in terms of pathogen kill without affecting seed vigour. Wakil XL could potentially be used as an alternative fungicide treatment, with an off-label approval already available for carrot and parsnip; however cheaper alternatives such as thiram and Jet 5 seem equally or more effective.

Promising results were obtained with Jet 5, both as a soak and also as a vapour treatment, although further studies would be needed to optimise application rates and treatment durations, to ensure that seed germination was not affected. Results from the vapour treatment

were particularly interesting since it has the advantage of not requiring seed-drying after treatment. Jet 5 is already available as a treatment for seed potatoes in conventional production, and these results suggest that there may be potential for further use of the product as a biocide for other seed-borne pathogens. Although it degrades into natural products, Jet 5 is unlikely to receive approval for treatment of organic seed since it is a manufactured, rather than a naturally occurring product.

Treatment with hot water (48^{o}C, 30 min) is the best available option for organic celery seed at present. Further discussion within the industry is needed to determine when this treatment will be applied, in relation to seed priming and pelleting.

Project conclusions

- Based largely on a knowledge review conducted in project year 1, a range of seed treatments were tested for their effects on seed-borne *Septoria apiicola* and celery seed germination:
  - hot water treatment
  - Disinfectants (Jet 5 and Vitafect)
  - microwave treatment
  - UV treatment
  - Essential oils (pine, *Eucalyptus citriadorus* and winter savory)
  - *Pythium oligandrum* (Polyversum®)
  - Fungicides (Wakil XL, Beret Gold and Thiram)

- A rapid method to determine *S. apiicola* spore germination (developed in project year 1), was used routinely to compare the effect of seed treatments on septoria levels.

- Excellent pathogen kill was obtained using a water pre-soak followed by hot water treatment (48ºC, 30 min) but there was also a reduction in seed germination. Hot water treatment (48ºC, 30 min) without a pre-soak, reduced *S. apiicola* spore germination to 3 % (compared with 34 % in the untreated control), without affecting seed germination tested immediately after treatment, and again after 8 months. The same treatment led to a reduction in the percentage of celery seeds with viable septoria infection (seeds releasing septoria spores that subsequently germinated) to 1 % compared with 18 % in the untreated control. Celery leaves inoculated with a suspension from hot-water treated seed did not develop septoria lesions, while 20 % of leaves inoculated with suspension from the untreated control seed developed typical septoria lesions with pycnidia.

- Hot water treatment (48ºC, 30 min) is the best option available for treatment of organic celery seed at present, although further discussion within the industry is needed to determine when this treatment will be applied, in relation to pelleting.

- Promising results were obtained with Jet 5 (5 % peroxyacetic acid), both as a soak and also as a vapour treatment, although further studies would be needed to optimise application rates and treatment durations, to ensure that seed germination was not affected. For example, 20 % Jet 5, reduced *S. apiicola* spore germination to 0.1 %, but had a deleterious effect on seed germination after storage (4 months). Particularly promising results were obtained with a Jet 5 vapour treatment, that would not require seed drying after treatment. There may be scope for further development of seed treatment using Jet 5, given that peroxyacetic acid is now approved as a commodity substance that can be used for treatment of surface-borne fungi on potato seed tubers prior to planting.

- Treatment with UV-A, UV-B and UV-C had negligible effect on septoria levels or celery seed germination, due largely to UV absorption by seed pigments.

- Microwave treatments of 120 sec or more reduced *S. apiicola* spore germination to approximately 10 % but also had deleterious effects on seed vigour. Reports in the literature of successful elimination of seed pathogens using microwaves, suggest that better results may be obtained using modified conditions.

- The essential oils and biological control agent (Polyversum®) used in these studies were ineffective in reducing seed-borne *S. apiicola*.

- The fungicide Wakil XL was effective in reducing *S. apiicola* spore germination without affecting seed germination. Wakil XL could potentially be useful as an alternative fungicide treatment, with an off-label approval already available for carrot and parsnip, however cheaper alternatives such as thiram and Jet 5 seem equally or more effective. Beret Gold had no effect on *S. apiicola* in these studies, although better results could probably be achieved with higher application rates.

- In a comparison of treatments with the industry standard, very promising results were obtained with hot water, Jet 5 (soak and vapour) and Wakil XL. However, the industry standard (warm water thiram soak) was still the most effective treatment in terms of pathogen kill without affecting seed vigour and was the only treatment that eliminated *S. apiicola*. However, all treatments reduced *S. apiicola* spore germination to less than 4 %. Soaking in 20 % Jet 5 was the only treatment to significantly reduce seed germination.

- The experiments carried out in this project looked largely at the effect of individual seed treatments on *S. apiicola* and celery seed germination and further studies to test treatment combinations may be warranted.

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Technology transfer

Meetings

- Project review meeting – 27.03.03
- Project progress meeting with D. Norman – 15.10.03
- Project review meeting, planned for 21.01.04

Technical advice

December 2003: Telephone advice and summary of project results provided to D. Norman and B. Lincoln as basis for ongoing industry discussions on organic seed treatment.

Article

Green, K. & O'Neill, T. 2004. Article for HDC News (submitted)

[Project results will also be included as appropriate in the Factsheet planned for related project FV 237, to be produced by March 2004].

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Appendix 1 – Experiment diaries

Hot water treatment

Date	Activity
29 November 2002	**Experiment 1:** Treatments applied to Batch 1 and 2 seed
02 December 2002	Batch 1 seed germination tests set up
04 December 2002	Septoria spore germination tests set up using Batch 2 seed
05 December 2002	Septoria spore germination recorded on Batch 2 seed
06 December 2002	Batch 1 seed germination tests transferred to sandwich boxes
16 December 2002	14 day seed germination count of Batch 1 seed
22 December 2002	20 day seed germination count of Batch 1 seed
21 January 2003	**Experiment 2:** Single treatment applied to Batch 1 seed – season 2002/3
17 April 2003	Season 2002/3 Batch 1 seed germination tests set up
30 April 2003	14 day seed germination count of Batch 1 seed
08 May 2003	21 day seed germination count of Batch 1 seed
21 January 2003	**Experiment 3:** Single treatment applied to Batch 1 and 2 seed – season 2001/2 Batch 1 seed stored
23 January 2003	Batch 2 seed Septoria spore release recorded – season 2001/2
24 January 2003	Batch 2 seed Septoria spore release recorded after 24h
27 February 2003	Pathogenicity test on healthy celery leaves set up – season 2001/2
12 March 2003	Pathogenicity tests assessed
3 September 2003	Batch 1 seed germination tests set up after 8 months storage
16 September 2003	13 day seed germination count of Batch 1 seed
25 September 2003	22 day seed germination count of Batch 1 seed

Disinfectant treatments

Date	Activity
24 March 2003	Treatments applied to Batch 1 and 2 seed
25 March 2003	Septoria spore germination tests set up
26 March 2003	Septoria spore germination recorded on Batch 2 seed
02 April 2003	Batch 1 seed germination tests set up
16 April 2003	14 day seed germination count of Batch 1 seed
24 April 2003	21 day seed germination count of Batch 1 seed
21 May 2003	Single treatment applied to Batch 1 and 2 seed
22 May 2003	Batch 1 seed stored
16 June 2003	Batch 2 seed Septoria spore release recorded after 0h
17 June 2003	Batch 2 seed Septoria spore release recorded after 24h
03 September 2003	Batch 1 seed germination tests set up after 4 months storage
16 September 2003	13 day seed germination count of Batch 1 seed
25 September 2003	22 day seed germination count of Batch 1 seed

UV treatments

Date	Activity
3 December 2002	UV treatments on batch 1 seeds completed
6 December 2002	Batch 1 seed germination tests set up
14 December 2002	UV treatments on batch 2 seeds completed
17 December 2002	Septoria spore germination tests set up using Batch 2 seed
18 December 2002	Septoria spore germination recorded on Batch 2 seed
20 December 2002	14 day seed germination count of Batch 1 seed

Microwave treatments

Date	Activity
20 November 2002	Experiment 1 – treatments applied to batch 1 seed
20 November 2002	Batch 1 seed germination tests set up
21 November 2002	Treatments applied to batch 2 seed
21 November 2002	Septoria spore germination tests set up (batch 2 seed)
22 November 2002	Septoria spore germination recorded (batch 2 seed)
04 December 2002	14 day seed germination count (batch 1 seed)
11 December 2002	21 day seed germination count (batch 1 seed)
21 January 2003	Experiment 2 – treatments applied to batch 1 seed
22 January 2003	Batch 1 seed germination tests set up
28 January 2003	Treatments applied to batch 2 seed
28 January 2003	Septoria spore germination tests set up (batch 2 seed)
29 January 2003	Septoria spore germination recorded (batch 2 seed)
5 February 2003	14 day seed germination count (batch 1 seed)
12 February 2003	21 day seed germination count (batch 1 seed)

Essential oil treatments

Date	Activity
22 April 2003	Treatments applied to Batch 1 and 2 seed
19 May 2003	Septoria spore germination tests set up using Batch 2 seed
20 May 2003	Septoria spore germination recorded on Batch 2 seed.

Polyversum seed treatment

Date	Activity
05 June 2003	Treatments applied to Batch 1 and 2 seed
09 June 2003	Batch 1 seed germination tests set up
09 June 2003	Batch 1 seed stored
16 June 2003	Batch 2 seed Septoria spore release recorded after 0h
16 June 2003	Septoria spore germination tests set up using Batch 2 seed
17 June 2003	Batch 2 seed Septoria spore release recorded after 24h
17 June 2003	Septoria spore germination recorded on Batch 2 seed
23 June 2003	14 day seed germination count of Batch 1 seed
12 September 2003	Batch 1 seed germination tests set up after 3 months storage
26 September 2003	14 day seed germination count of Batch 1 seed
29 September 2003	Septoria spore germination tests set up using Batch 2 seed (repeat)
30 September 2003	Septoria spore germination recorded on Batch 2 seed (repeat)
03 October 2003	21 day seed germination count of Batch 1 seed

Fungicide treatment

Date	Activity
21 July 2003	2 x 20g of Batch 1 and 2 seeds were sent to Andy Jukes, HRI
04 August 2003	Treated seed received
04 August 2003	Batch 1 seed stored
11 August 2003	Batch 1 seed germination tests set up
	Septoria spore germination tests set up using Batch 2 seed
12 August 2003	Septoria spore germination recorded on Batch 2 seed
	Batch 2 seed Septoria spore release recorded at 0h
13 August 2003	Batch 2 seed Septoria spore release recorded after 24h
26 August 2003	14 day seed germination count of Batch 1 seed
29 September 2003	Septoria spore germination tests set up using Batch 2 seed (repeat)
30 September 2003	Septoria spore germination recorded on Batch 2 seed (repeat)
12 November 2003	Batch 1 seed germination tests set up
27 November 2003	15 day seed germination count of Batch 1 seed
03 December 2003	21 day seed germination count of Batch 1 seed

Comparison with industry standard

Date	Activity
14-15 October 2003	Treatments applied to Batch 1 and 2 seed
16 October 2003	Septoria spore germination tests set up
17 October 2003	Septoria spore germination recorded on Batch 2 seed
21 October 2003	Batch 1 seed germination tests set up
04 November 2003	15 day seed germination count of Batch 1 seed
12 November 2003	22 day seed germination count of Batch 1 seed