Protected ornamentals: detection, prevalence and control of seed-borne diseases

PC 252

June 2008

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Horticultural Development Company
Stable Block
Bradbourne House
East Malling
Kent
ME19 6DZ

Tel: 01732 848 383
Fax: 01732 848 498

Project title:	Protected ornamentals: detection, prevalence and control of seed-borne diseases
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Project leader:	Dr T M O'Neill ADAS Boxworth Boxworth Cambs CB23 4NN
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Key workers:	Dr K Green, Ms A Shepherd. Ms H Greenleaves, Ms T Adamowicz, Ms L Kirkpatrick, ADAS Mr J Scrace, Consultant Pathologist Dr G M McPherson, Ms C Lambourne, Miss I Burdon, Miss D Liddell, STC Mr S Coutts – Consultant to project
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Project co-ordinator:	Ms F Richardson Coletta & Tyson
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The results and conclusions in this report are based on experimental work conducted over one year. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

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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 T M O'Neill
Principal Research Scientist
ADAS Boxworth

Signature ..... Date .....

Ms C Lambourne
Project Manager
Stockbridge Technology Centre

Signature …….. Date

Report authorised by:

Dr W E Parker
Horticulture Sector Manager
ADAS Wolverhampton

Signature ..... Date .....

Dr G M McPherson
Science Director
Stockbridge Technology Centre

Signature ..... Date .....

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GROWER SUMMARY

Headline

Tests of 25 commercial seed lots of 15 ornamental species recovered several pathogens that commonly affect ornamentals crops but after treatment with sodium hypochlorite, no fungi or bacteria developed from over 80% of seeds in most batches. Other seed and seedling treatments that warrant evaluation on ornamentals have been identified.

Background and expected deliverables

Seed-borne diseases occur sporadically on a wide range of ornamental crops resulting in substantial and widespread crop losses, disruption to production schedules and increased use of pesticides. The true impact of seed-borne pathogens on the UK industry may be greater than is commonly appreciated, due to the uncertain nature of disease origin. For example, where pathogens are present in seed at a low level or disease development is slow, a disease outbreak may be wrongly attributed to an infection source other than the seed. The aims of this project are to:

- Prepare a list of reported seed-borne diseases of major ornamental species grown in the UK and the risks they pose
- Determine appropriate testing methods for important crop-pathogen combinations and record, over three seasons, the levels of pathogens on commercial lots of different cultivars of 10 key ornamentals
- Recover suspect fungal and bacterial pathogens from seed-lots and determine their pathogenicity
- Identify and test promising chemical and non-chemical treatments for control of seed-borne pathogens
- Summarise information relevant to growers in an illustrated factsheet. Increased knowledge on the occurrence and control of seed-borne pathogens should ultimately result in reduced losses to disease and sustained production of high quality crops.

Summary of the project and main conclusions

Prevalence of seed-borne fungi and bacteria

Twenty-five lots of ornamental seeds were tested for occurrence of fungi and bacteria by plating them onto selective agar media. Seeds were tested both directly and after surface disinfection in sodium hypochlorite.

From the seed batches tested by ADAS, a range of saprophytic fungi (e.g. *Cladosporium* sp., *Penicillium* sp., *Mucor* sp. were recovered from most of the non-disinfected seeds of most samples tested. The exceptions were geranium and lupin, where over 70% of seeds not treated with sodium hypochlorite were 'clean' (i.e. there was no fungal or bacterial growth from the seeds). After treatment with sodium hypochlorite, no fungi or bacteria developed from over 80% of seeds in most batches. Suspect pathogens were recovered from alyssum (*bacteria*), cineraria (*Alternaria* sp.), coreopsis (*Alternaria* sp.), cyclamen (*Fusarium oxysporum*), lychnis (*Phoma* sp.), tagetes (*Alternaria* sp.) and zinnia (*Alternaria* sp). Two batches of lupin seeds were tested and no *Colletotrichum acutatum* was recovered from either batch. Isolates of bacteria from alyssum, *F. oxysporum* from cyclamen, *Phoma* sp. from lychnis and *Alternaria* sp. from various hosts were maintained for pathogenicity tests.

Of the seed batches tested to date at STC, the lupin, lobelia and geranium seeds have proved to be the 'cleanest' with no fungal or bacterial growth on >70% of the seed plated without surface disinfection. Conversely, high numbers of bacterial and fungal organisms were recovered from the zinnia and tagetes seed even following surface sterilisation. A similar range of saprophytic organisms were detected on the STC seed batches as were observed during the ADAS tests with the notable additions of *Phomopsis* on tagetes and low levels of *Curvularia* sp. on zinnia. *Colletotrichum acutatum* was not recovered from the batch of lupin seed tested. Very high levels of *Alternaria* sp. were recovered from zinnia.

Pathogenicity tests

Pathogenicity of bacteria to alyssum

An isolate of *Bacillus* sp. recovered from alyssum seed caused no symptoms when inoculated onto alyssum seedlings. Isolates of a *Pseudomonas* Group IVb and unidentified bacteria from rotting leaves of alyssum seedlings also caused no symptoms when inoculated onto alyssum seedlings. From examination of other samples of alyssum seedlings with leaf damage, it is suggested that the leaf damage and collapse of seedlings seen in alyssum in recent years is, at least in some instances, due to development of an oedema in leaves followed by colonisation of the damaged leaves by non-pathogenic bacteria.

Other bacterial pathogenicity tests

Isolates of bacteria recovered from antirrhinum, pelargonium, salvia and tagetes did not cause disease symptoms when inoculated on to seedlings of the host from which they were isolated.

Pathogenicity of B. streptothrix to lobelia

Although lobelia plants inoculated with *Botrytis streptothrix* (isolated from lobelia seed in 2006) developed a botrytis stem rot, the incidence of affected plants was low and similar to that found in uninoculated plants. An isolate of *B. cinerea* was more damaging to the lobelia. It was concluded that *B. streptothrix* is not strongly pathogenic to lobelia.

Other fungal pathogenicity tests

Isolates of *Alternaria* and *Fusarium* obtained from seed of senecio and zinnia did not cause disease symptoms when inoculated on to seedlings of the host from which they were isolated.

Review of treatments for control of seed-borne diseases

Published reports of seed treatments that could be of potential use in the treatment of seed-borne diseases of ornamentals are summarised below.

Fungicides

A large number of fungicide products are available for seed treatment of arable crops against a range of fungal pathogens. A few products are also approved for use on outdoor horticultural vegetable crops e.g. Wakil XL (cymoxanil, fludioxonil and metalaxyl-M). Some of these could have potential for use against seed-borne pathogens of ornamentals. Currently, the only fungicide with an on-label recommendation for use as a seed treatment on ornamental plants is iprodione. This fungicide has activity against fungi such as *Alternaria, Botrytis* and *Rhizoctonia* though problems with fungicide resistance are known to occur in *B. cinerea* and *A. alternata*. Thiram can also be used as a seed treatment on some ornamental crops (e.g. lupin) under Specific Off-Label Approval (SOLA) 2394/05. The Long Term Arrangements for Extension of Use (LTAEU) may allow extrapolation of seed treatments to ornamentals from edible crops. However, these arrangements are currently under review and will be phased out and substituted with individual SOLA documents, subject to specific notification of the use (via HDC/PSD).

It is important to note here that seeds of some ornamental species (e.g. alyssum, *Salvia splendens*, viola) have a natural gelatinous coating and when wetted up (i.e. with a seed soak) they cannot then be drilled using automated machinery. Un-rubbed seed of marigold and zinnia can also cause problems because of hairiness. Very small seed are difficult to treat by chemicals because of their small surface area. Therefore, considerable care must be taken when considering any ‘experimental’ treatments of seed.

Storage time

Whilst levels of seed-borne diseases might be expected to decline with increasing length of seed storage, there are also usually adverse effects on the viability of the seed itself. Conversely, conditions suitable for maintaining seed viability in long-term storage are often also conducive to maintaining the viability of seed-borne pathogens. Research on this technique for control of seed-borne diseases often gives variable results, probably because of differences in the storage conditions used by different researchers. One report states that 'unless the storage parameters of temperature and relative humidity have been thoroughly tested against seed-borne inoculum, the policy of ageing seed to free it of seed-borne fungi may not be fully effective and therefore is of doubtful value'.

Thermotherapy

Thermotherapy can involve the use of hot water, aerated steam or dry heat treatments. Hot water has been used since the 1920s and, before the advent of systemic fungicides in the 1960s, was the only treatment available to eradicate deep-seated infections of seed. Water is twice as effective for heat transfer as steam and five times as effective as dry heat. However, seed needs to be dried after hot water treatment, and there may be problems with damage to the seed coat and reduced germination.

Aerated steam treatment causes less damage to the seed, and there is no need for the seed to be dried after treatment. The process is now available as a commercial system in Sweden for the treatment of cereal seed (Thermoseed®). Due to variation in sensitivity to treatment between seed lots, representative samples are laboratory-tested and the precise requirements determined from mathematical models prior to the steam treatment. This technique is considered to have high potential for practical use for horticultural crop seeds. Generally, for both hot water and aerated steam treatment, more mature seeds are less prone to damage than less mature seeds.

Dry heat treatment is less damaging to seed than either hot water or aerated steam, but the high temperatures and long exposures required mean that there are fire and safety hazards to consider.

UV radiation

UV radiation is that part of the electromagnetic spectrum between 200 and 400 nm, and is conventionally divided into three components (UV-A, UV-B and UV-C). UV radiation is still at an early stage of development as a seed treatment.

Microwave treatment

Microwaves have been used to eradicate both surface-borne and more deep-seated infection on seeds and planting material of a diverse range of crops. The duration and power of the treatment required varies between plant species. The effect on seed germination can also vary according to both seed size and seed moisture content. Adverse effects are reduced when smaller seeds or those with lower moisture content are treated.

Whilst microwave treatment had adverse effects on seed germination of celery and variable efficacy against *Septoria apiicola*, the use of microwaves on a range of other crops has given good results against both surface-borne and more deep-seated infection. For example, microwave treatment was used to eradicate seed-borne pathogens (including *Fusarium* spp., *Cladosporium, Colletotrichum, Diplodia* and *Xanthomonas campestris* pv. *manihotis*) in cassava true seed. The efficacy of microwave treatment against seed-borne pathogens is relatively straightforward to evaluate and potentially treatment could be undertaken by growers.

Other radiation methods

Gamma radiation is a well-known technique used in food preservation. Other radiation methods that have been tested for seed treatment include the use of solar heat, laser treatments and radiowaves. None are used widely on a commercial scale, if at all.

Low energy electron treatment is another technique, which is utilised successfully in Germany for cereal seed treatment as the e-ventus® static or mobile systems. In general, hot water and aerated steam treatments were found to be more effective than electron treatment when used on a range of seed-borne vegetable pathogens.

Disinfectants

Disinfectants represent a cheap alternative where elimination of surface-borne fungi from seed is required. Their usefulness for eradication of more deep-seated infection is unclear, although pre-soaking seeds may increase their effectiveness. However, commercial disinfectants cannot be used for seed treatment unless they are specifically approved for this purpose. Use of disinfectants is regulated by the Biocides Directive (EC Directive 2032/2003) and, in the UK responsibility lies with the HSE (www.hse.gov.uk/biocides).

Plant extracts and other 'natural' products

The anti-fungal properties of essential oils have been reported in research publications, and their use in the liquid or vapour phase may have potential for seed treatment. The use of

plant oils is permitted in organic agriculture under EU regulation 2092/91. Oils that have been evaluated against a range of crop / pathogen combinations include those from thyme, marjoram, dictamnus and eucalyptus. Other 'natural' products reported to have anti-fungal properties include mustard, flour and milk powder.

Biological control

There are numerous reports of potentially valuable biological control micro-organisms, some of which are supplied as seed treatments. However, there can be numerous problems in the development of biological control agents (BCAs) into commercial products due to factors such as formulation difficulties, shelf-life and storage problems, erratic biological efficacy and the economic viability given the various regulatory constraints, especially within the EU.

Pythium oligandrum and *Coniothyrium minitans* are mycoparasites developed for use against a range of damping-off pathogens (e.g. *Pythium* and *Rhizoctonia* species) and *Sclerotinia sclerotiorum*. A commercial preparation of *Pythium oligandrum* is available in some countries (but not the UK) as Polyversum®, with recommendations for seed treatment. Contans®, a product containing *Coniothyrium minitans,* is available in the UK, but for soil treatment only (against *Sclerotinia*). Mycostop®, a commercial product containing *Streptomyces griseoviridis*, is available in many countries (but again, not in the UK) and also has recommendations for seed treatment of ornamentals and other crops. The product claims control of *Fusarium, Alternaria* and *Phomopsis,* with suppression of *Botrytis, Pythium* and *Rhizoctonia*. Some resistance-inducing compounds have been evaluated as potential seed treatments but results to date have not been promising.

Seed and seedling treatments that we consider warrant evaluation on ornamentals are summarised below.

Table 1: Summary of potential seed and seedling treatments for evaluation on ornamentals

Treatment method (and potential provider)	Example product	Active substances in product	Example target	Priority
Fungicide seed treatment (by seed treatment company)	Wakil XL	Cymoxanil + fludioxonil + metalaxyl-M	*Botrytis, Septoria*	Medium
	Hy-TL	Thiabendazole + Thiram	*Botrytis, Colletotrichum, Phoma*	High
Fungicide seed soak (by grower)	Thiram	Thiram	*Botrytis, Septoria, Colletotrichum*	High
Fungicide seedling spray (by grower)	Cercobin Liquid	Thiophanate-methyl	*Botrytis*	High
	Octave	Prochloraz	*Botrytis, Fusarium*	High
	Signum	Pyraclostrobin + boscalid	*Botrytis, Phoma*	High
Hot water seed treatment	-	-	*Alternaria, Botrytis,*	High

Treatment method (and potential provider)	Example product	Active substances in product	Example target	Priority
(by grower)			bacteria	
Aerated steam seed treatment (by specialist company)	-	-	*Various*	High[+]
Chemical disinfection of seed (by grower)	Jet 5	Peroxyacetic acid	*Botrytis, Septoria*	Low*
Plant extracts	Thyme oil	Oils	*Various*	Medium
Biocontrol agents	Mycostop	*Streptomyces griseoviridis*	*Fusarium*	Low*
UV treatment	UV-C	-	Bacteria	Low
Microwave treatment (by grower)	-	-	*Colletotrichum, Fusarium*	Medium

* Rated as low priority due to probable difficulty in securing approval for use in the near future.
[+] Dependent on cooperation of seed treatment company and economic evaluation on viability of such a service.

The approval of Rovral WP (iprodione) as a seed treatment for ornamentals is due to be transferred to Rovral Aquaflo. This new fungicide will be considered for evaluation if the approval is gained during the life of this project.

Financial benefits

Increased knowledge on the occurrence and control of seed-borne pathogens should ultimately result in reduced losses to disease and sustained production of high quality crops. The farm-gate value of bedding plant production in the UK is estimated at more than £250 million (S. Coutts, pers. comm.). Many of the most important subjects (impatiens, lobelia, geranium, antirrhinum, salvia, nicotiana, nemesia) are affected, from time-to-time, by seed-borne diseases. If just 1% of production is lost, this represents £2.5 million per annum.

Action points for growers

- Growers should be aware of the potential seed-borne origin of key diseases of important pot and bedding plant species as detailed in Table 1 of the first year report.
- For species commonly affected by seed-borne pathogens, examine plants for disease at an early growth stage. Take action promptly to control any diseases found (e.g. lupin anthracnose).

SCIENCE SECTION

Introduction

Each year a number of diseases that are known to be seed-borne cause significant losses in ornamental crops produced in the UK. Some problems occur virtually every year (e.g. lupin anthracnose caused by *Colletotrichum acutatum*; lobelia leaf blight caused by *Alternaria* spp., cineraria leaf spot caused by *Alternaria cinerariae*; cyclamen fusarium wilt caused by *Fusarium oxysporum*), while others occur more sporadically (e.g. *Xanthomonas campestris* in wallflower; leaf spots on antirrhinum and salvia caused by *Pseudomonas syringae*). Occasionally pathogens new to the UK are believed to have been introduced on seed and/or vegetative transplants (e.g. impatiens downy mildew caused by *Plasmopara obducens*).

The number of known seed-borne diseases is large with fungal diseases the most common, especially those caused by species of *Alternaria*, *Botrytis*, *Colletotrichum, Septoria, Phoma* and *Fusarium*. Bacterial seed-borne diseases are important on certain species. Contamination of seed-lots with fungal sclerotia (e.g. *Sclerotinia sclerotiorum*) can also occur. Grower and propagator knowledge of the occurrence of pathogens on seeds is limited.

Seed may be contaminated with pathogens in a variety of ways including surface contamination, deep-seated (e.g. endosperm) infection, and trash-borne contamination, including sclerotia of *Sclerotinia* species.

Production of ornamental seeds is a global business increasingly centred on Africa and China. Information on locations and conditions of seed production, on the nature of any seed treatments applied and any testing for pathogens undertaken prior to sale largely remain confidential to the seed companies. There may be an increased risk of introducing non-indigenous pathogens via the seed where seed crops are grown in distant countries where the prevalence of particular pathogens may be unknown. There is no public domain information on the occurrence of plant pathogens recently found on ornamental seeds used by UK growers.

Government, retailers and consumers demand sustainable production with minimal use of pesticides. When a disease outbreak on a nursery originates on seed, the most efficient and effective method of control, requiring minimal use of pesticides, is by an appropriate seed treatment. In some instances physical treatments (e.g. heat, hot air) are effective. Where a non-chemical method of control is unavailable, a single chemical treatment of the seed may

result in satisfactory control, eliminating the need for routine fungicide applications during crop production. In addition to savings on costs, an effective seed treatment has minimal adverse impact on the environment, poses little health risk to nursery staff and helps to avoid fungicide resistance problems, in comparison with a series of fungicide sprays. It is accepted that some seeds are difficult to treat due to the gelatinous (slimy or sticky) nature of the seed when wet.

This project aims to inform propagators and growers of the key seed-borne fungal and bacterial pathogens of ornamentals, to ascertain the current prevalence of plant pathogens on seeds of major ornamental species, and to determine the effectiveness of chemical and non-chemical treatments, including novel approaches, in reducing disease outbreaks.

In year 1 of this project, a comprehensive listing was done of the bedding and pot plant diseases that can originate from use of infected seeds. Out of 29 commercial seed lots of 18 ornamental species tested, the only pathogens recovered were *Botrytis cinerea* (10 seed lots), *Alternaria* species (5 seed lots), *Colletotrichum acutatum* (1 seed lot) and a *Pythium* species (1 seed lot).

In year 2 of the project, the objectives are to:

- Determine the levels of seed-borne fungi on up to 30 commercial seed lots;
- Test the pathogenicity of selected fungi isolated from seeds by inoculation of young plants;
- Review the literature on chemical and non-chemical methods for control of seed-borne pathogens, and select promising treatments for use in this project;
- Test potential chemical and non-chemical treatments on a selection of seed-borne diseases, using naturally infected seed if available, otherwise artificially contaminated seed.

Prevalence of seed-borne fungi and bacteria

Introduction

Seeds were obtained from commercial propagators, predominantly as unopened packets of species being grown in their nurseries. The aim was to determine the incidence of seed-borne pathogens on the seeds, and to monitor the occurrence of seed-borne diseases in seedlings grown from these batches of seed.

Materials and methods

The methods described in the Year 1 report were followed.

Results and discussion

A total of 15 seed lots were tested by ADAS and a further 15 by STC (completed results available for 10). Results of the samples tested by ADAS are shown in Table 1. A range of saprophytic fungi (*Cladosporium* sp., *Penicillium* sp., *Mucor* sp.) were also recovered from most of the non-disinfected seeds of most batches tested. The exceptions were geranium and lupin, where over 70% of seeds were 'clean' (i.e. there was no fungal or bacterial growth from the seeds) even before treatment with sodium hypochlorite. Possibly this indicates that these seed were treated in some way for control of potential pathogens before packing. After treatment with sodium hypochlorite, over 80% of seeds were 'clean' in most batches. An exception was a seed lot of cineraria for which the incidence of bacterial species was 44% on surface sterilised seed. Suspect target pathogens were recovered from the following: antirrhinum (*Pseudomonas syringae*), alyssum (bacterial spp.), cineraria (*Alternaria* sp.) coreopsis (*Alternaria* sp.), cyclamen (*Fusarium oxysporum*), geranium (bacterial spp.), lobelia (*Alternaria* sp.), lychnis (*Phoma* sp.), senecio (*Alternaria* sp.), tagetes (*Alternaria* sp.) and zinnia (*Alternaria* sp. and *Botrytis cinerea*). Two batches of lupin seeds were tested and *Colletotrichum acutatum* was not recovered from either batch. Isolates of bacteria from alyssum, *F. oxysporum* from cyclamen, *Phoma* sp. from lychnis and *Alternaria* spp. from various hosts were maintained for pathogenicity tests. Despite the absence of the target pathogen(s) for some ornamental species, other possible pathogens were isolate. For example, a *Stemphylium* sp. was isolated from 19% of non-surface sterilised pansy seeds and 11% of cineraria seeds were infested with a *Fusarium* sp.

The seed testing at STC is still on-going following a late start due to seed batch delivery delays. Results of the seed testing are shown in Table 2. As with the seed batches tested by ADAS a variety of saprophytic fungi (*Cladosporium* sp. *Penicillium* sp., *Mucor* sp. and

Fusarium sp.) were detected from most of the non-disinfected seed and also some disinfected seed. The lobelia, lupin and geranium seed batches tested to date have proved to be quite ‘clean’ with only low levels of saprophytic organisms present on the non-surface sterilised seed. Suspect pathogens were recovered albeit at low levels from lobelia (*Alternaria* sp.) and possibly from tagetes (bacteria recovered, awaiting pathogenicity testing). High numbers of zinnia seed were found to be carrying *Alternaria* sp. with similar levels found on both sterilised and non-sterilised seed.

Table 1: Recovery of fungi and bacteria from ornamental seeds - ADAS tests, Year 2

	Crop	Target plant pathogen(s)	% of seeds from which target plant pathogens were recovered (300 seed tested)		% of 'clean' seeds[c]	
			Surface sterilised	Not surface sterilised	Surface sterilised	Not surface sterilised
1.	Alyssum	*Pseudomonas syringae*	14.3	66.3	77.0	0.0
2.	Alyssum	*Pythium*	0.0	0.0	0.0	0.0
3.	Cineraria	*Alternaria cinerariae*	0.0	5.0	55.0	0.7
4.	Coreopsis	*Alternaria* sp.	23.7	66.0	27.0	0.0
5.	Cyclamen	*Fusarium oxysporum*[a]	0.0	14.0	96.7	20.3
6.	Geranium	*Pseudomonas* and *Xanthomonas spp.*	NT	0.0	NT	74.7
7.	Lupin 1.	*Colletotrichum acutatum*	0.0	0.0	85.0	85.3
8.	Lupin 2.	*Colletotrichum acutatum*	0.0	0.0	97.3	98.7
9.	Lychnis	*Phoma* sp.	6.7	53.0	56.0	0.0
10.	Pansy	*Ramularia lactis* and *R. agrestis*	0.0	0.0	98.7	6.0
11.	Primula	*Botrytis cinerea*	0.0	0.0	98.0	0.0
12.	Tagetes 1.	*Alternaria alternata*	9.1	16.0	83.6	0.0
13.	Tagetes 2.	*Alternaria alternata*	12.4	22.8	80.4	0.0
14.	Zinnia 1.	*Alternaria* sp.[a,b]	12.0	0.0	66.0	12.0
15.	Zinnia 2.	*Alternaria* sp.[a,b]	20.3	5.3	45.3	2.0

Notes:

[a] *Botrytis cinerea* also sought on cyclamen and zinnia and none found.

[b] Zinnia - 50 seed tested in batch 501; 300 seed in batch 502

[c] 'Clean seed' - no pathogens or saprophytes recovered.

Use of selective agar media for target plant pathogens may result in failure to detect some non-target plant pathogens.

NT - not tested.

Table 2: Recovery of fungi and bacteria from ornamental seeds - STC tests, Year 2

	Crop	Target plant pathogen(s)	% seeds from which target plant pathogens recovered (250 seeds tested)		% of 'clean' seeds (of 250)[a]	
			Surface sterilised	Not surface sterilised	Surface sterilised	Not surface sterilised
1.	Antirrhinum	*Pseudomonas syringae*	0.4	0.8	99.2	31.2
2.	Aquilegia	*Alternaria* sp., *Pythium* sp.	On-going	On-going	On-going	On-going
3.	Cyclamen	*Fusarium oxysporum*	0.0	0.0	99.6	90.0
4.	Geranium	*Pseudomonas & Xanthomonas* spp.	2.4	1.6	97.6	88
5.	Lobelia	*Alternaria alternata* & *Sclerotinia sclerotiorum*	0.0 0.0	0.7 0.0	99.7	96.7
6.	Lupin	*Colletotrichum acutatum*	0.0	0.0	85.6	81.6
7.	Pansy	*Ramularia lactis & R. agrestis*	On-going	On-going	On-going	On-going
8.	Petunia	*Sclerotinia* spp.	On-going	On-going	On-going	On-going
9.	Primula	*Botrytis cinerea*	0.0	0.0	33.6	2.8
10.	Rudbeckia	*Alternaria* spp.	On-going	On-going	On-going	On-going
11.	Salvia	*Pseudomonas* sp.	7.6 ^(bacteria)	59.6 ^ (bacteria)	76.4	14.0
12.	Senecio	*Alternaria* sp. & *Botrytis cinerea*	4.4 (*Alternaria*)	3.6 (*Alternaria*)	90.0	82.0
13.	Tagetes	*Alternaria, Rhizoctonia & Pseudomonas*	1.2^	0.0	84.4	10.8
14.	Verbena	*Alternaria* sp. *Phoma*	On-going	On-going	On-going	On-going
15.	Zinnia	*Alternaria* sp. *& Botrytis cinerea*	74.8 (*Alternaria*) 0.8 (*Botrytis*)	75.6 (*Alternaria*)	3.2	0

Notes:

[a] 'Clean seed' – no pathogens or saprophytes recovered.

Use of selective agar media for target plant pathogens may result in failure to detect some non-target plant pathogens.

* Suspect colonies only- awaiting confirmation

^ Bacteria, awaiting pathogenicity test

Pathogenicity tests of fungi recovered from seed

Introduction

Certain fungi and bacteria detected on seed were tested to determine if they were pathogenic to the crop from which they were isolated. This was particularly important for *Alternaria* and bacterial species, as both saprophytic and pathogenic species may occur on seed.

Materials and methods

Botrytis streptothrix inoculation of lobelia

In May 2007, leaves and stems of lobelia grown in 9 cm pots were inoculated with mycelium of *B. streptothrix* and *B. cinerea* on PDA discs (5 mm diameter). Control plants were inoculated with PDA alone. There were five plants per treatment. Plants were enclosed in a polythene bag for 24 h to maintain a high humidity, and grown on in a shaded, cool glasshouse (15–20°). Plants were examined for leaf and stem rot at 7, 14 and 28 days after inoculation.

Bacterial inoculation of alyssum

In June 2007, leaves of alyssum 'Snowdrift' (3 per plant) were stab-inoculated with four bacterial types isolated from alyssum seed (AR07/51(1), AR07/51(2), AR07/51(3) and AR07/60, and with sterile distilled water (SDW control). There were five replicate plants per treatment. Plants were incubated in a polythene bag for 4 days after inoculation then grown on in a warm glasshouse (20–25°). Plants were examined for symptoms after 14 and 21 days. Five bacterial isolates obtained from rotting alyssum leaves were also tested.

Phoma sp. inoculation of lychnis

Ten leaves on a pot-grown plant of lychnis were inoculated with mycelial discs (5 mm diameter) of a *Phoma* sp. isolated from lychnis seeds, on potato dextrose agar amended with streptomycin (PDA+S). Plugs of PDA+S only were placed on five different leaves as uninoculated controls. Plants were examined for symptoms after 7, 14 and 28 days.

Bacterial inoculations by STC

Pathogenicity tests with a range of bacteria isolated from antirrhinum, pelargonium, salvia and tagetes seed were set up in May 2008. Bacteria from the cultures were suspended in sterile distilled water and used to inoculate damaged and undamaged seedlings with three replicates/isolate. Uninoculated damaged and undamaged control plants along with the

inoculated plants were grown-on in germination boxes. Plants were examined daily and were retained for 3 weeks. Additional tests on aquilegia are still in progress.

Fungal inoculation by STC

Pathogenicity tests with fungal isolates collected from the initial seed testing were also carried out. Isolates of *Alternaria* spp. were tested on zinnia and senecio seedlings, and a *Fusarium* isolate was tested on zinnia. Batches of seedlings were inoculated by spraying with a spore solution of the fungus being tested with additional plants being inoculated by placing a piece of agar + fungus on the leaf. Damaged and undamaged seedlings were used and tests plants were compared to uninoculated damaged and undamaged seedlings.

Additional pathogenicity tests on verbena and aquilegia seedlings are still in progress.

Results

Pathogenicity of bacteria to alyssum

An isolate of *Bacillus* sp. recovered from alyssum seed caused no symptoms when inoculated onto alyssum seedlings. Isolates of a *Pseudomonas* Group IVb and unidentified bacteria from rotting leaves of alyssum seedlings also caused no symptoms when inoculated onto alyssum seedlings. From examination of other samples of alyssum seedlings with leaf damage, it is suggested that the leaf damage and collapse of seedlings seen in alyssum in recent years is, at least in some instances, due to development of an oedema in leaves followed by colonisation of the damaged leaves by non-pathogenic bacteria. See Table 3.

Pathogenicity of B. streptothrix to lobelia

Although lobelia plants inoculated with *Botrytis streptothrix* (isolated from lobelia seed in 2006) developed a botrytis stem rot, the incidence of affected plants was low and similar to that found in uninoculated plants. An isolate of *B. cinerea* was more damaging to the lobelia. It was concluded that *B. streptothrix* is not strongly pathogenic to lobelia. See Table 3.

Pathogenicity of Phoma sp. to lychnis

No symptoms developed when mycelial discs of *Phoma* sp. were applied to lychnis leaves. A modified pathogenicity test using spray inoculation of lychnis plants will be done once mature pycnidia (with spores) of *Phoma* sp. *ex* lychnis seed have formed on culture plates. See Table 3.

Bacterial inoculations by STC

No lesions developed on any of the seedlings inoculated with the bacterial isolates collected from the seed batches tested. It was concluded that the bacteria were not pathogenic and no further identification of the collected isolates e.g. fatty acid profiling was carried out in year *2* (Table 4).

Fungal inoculations by STC

None of the fungal organisms isolated and inoculated onto seedlings (e.g. *Fusarium* and *Alternaria* spp.) resulted in the development of lesions on seedlings of the same species. A number of tests are still on-going (see Table 4).

Table 3: Pathogenicity of fungi and bacteria isolated from various ornamental seeds and seedlings to young plants of the same species from which they were isolated (ADAS)

Plant species	Organism	Isolated from	ADAS code	Symptoms observed	Proportion of plants affected
Alyssum	*Bacillus* sp. [a]	Alyssum	AR07/60	None	0/20
	Uninoculated	-	-	None	0/20
	Unidentified bacteria (x5)	Alyssum	AR07/23	None	0/20
	Pseudomonas Gp IV [b] (x3)	Alyssum	AR07/51	None	0/20
	Uninoculated	-	-	None	0/20
Lobelia	*B. streptothrix*[c]	Lobelia		Dead stems	6/15
	B. cinerea	Poinsettia	AR03/133	Dead stems	12/15
	Uninoculated	-	-	Dead stems	4/15
Lychnis	*Phoma* sp.	Lychnis	BX08/07	Mycelium: nil	0 leaves of 10
	Phoma sp.	Lychnis	BX08/07	Spores: nil	Ongoing
	Uninoculated	-	-	-	-

[a] Not known to be plant pathogens. This isolate was tentatively identified by CSL as *B. megaterium.*
[b] Isolated from rotting leaves of alyssum, cv. Snowdrift.
[c] Culture maintained and tested under Defra Plant Health licence 256/5445 (2006).

Table 4: Pathogenicity of fungi and bacteria isolated from various ornamental seeds and seedlings to young plants of the same species from which they were isolated (STC)

Plant species	Organism	Isolated from	Symptoms observed	Proportion of plants affected
Antirrhinum	Uninoculated	-	None	0/20
	Unidentified bacteria (x3)	Antirrhinum	None	0/20
Pelargonium	Uninoculated	-	None	0/20
	Unidentified bacteria (x4)	Pelargonium	None	0/20
Tagetes	Uninoculated	-	None	0/20
	Unidentified bacteria (x2)	Tagetes	None	0/20
Salvia	Uninoculated	-	None	0/20
	Unidentified bacteria (x8)	Salvia	None	0/20
Senecio	Uninoculated	-	None	0/20
	Alternaria sp.	Senecio		
Zinnia	Uninoculated	-	None	0/20
	Fusarium sp. (x2)	Zinnia	None	0/20
	Alternaria sp.	Zinnia	None	0/20
Aquilegia	Uninoculated	-	In progress	
	Unidentified bacteria (x5)	Aquilegia	In progress	
	Alternaria sp.	Aquilegia	In progress	
Verbena	Uninoculated	-	In progress	
	Alternaria spp. (x3)	Verbena	In progress	

Evaluation of seed and seedling treatments

Introduction

At the project review meeting held on 31 May 2007, it was agreed that the targets for seed and seedling treatments should be pathogens found on seed in project year 1 and known to cause disease in UK crops. It was suggested that an appropriate range of organisms was:

Alternaria sp. (e.g. on zinnia)

Botrytis cinerea (e.g. on pansy, pelargonium)

Colletotrichum acutatum (e.g. on lupin)

A bacterial pathogen (e.g. on wallflower)

Materials and methods

Attempts to locate large batches of seed (sufficient for experimental seed treatments) that were known to be naturally infested by one or more of the above pathogens were unsuccessful. Work on this objective will continue in year 3.

Review of chemical and non-chemical methods for control of seed-borne pathogens

Introduction

The aim of this short review was to collate the results of recent studies on chemical and non-chemical methods of controlling seed-borne diseases on any crop and identify methods which appear to have the greatest potential. A number of the identified treatments will then be evaluated for control of seed-borne diseases of ornamentals.

A review of scientific literature was done using a web search to identify potentially useful papers. Additionally, the results of recent HDC projects on control of seed-borne diseases were examined, results of the EU STOVE project on seed-borne diseases were examined and discussed with Dr Steve Roberts (participant), and the standard text on seed-borne diseases and their control (Maude, 1996) was examined.

Research into seed treatments against pathogens of ornamental plants

Antirrhinum

Dry heat for 8 hours at 49°C destroyed the bacterial leaf spot pathogen *Pseudomonas syringae* pv. *antirrhini* on seed, but also tended to reduce germination (Simpson *et al.,* 1971).

Lavatera

HDC-funded research into the control of seed-borne *Colletotrichum* species, the cause of anthracnose disease on both *Lavatera* and lupin, was carried out by Maude (1994). It was found that infection was present in the seed coat, endosperm and embryo, meaning that to be effective any seed treatments had to penetrate the seed tissues. Laboratory tests showed that prochloraz; prochloraz + carbendazim, thiram + benomyl, thiram and thiabendazole (applied at 1 g a.i. / kg of seed) all eradicated infection without affecting germination.

Lobelia

Good control of the leaf spot and stem rot pathogen *Alternaria alternata* was obtained with an aerated steam treatment at 50-51°C for 15-20 minutes (Hall & Taylor, 1993). Thiram used as seed soak was ineffective and reduced germination. Iprodione is also used as a seed treatment, but there have been problems with fungicide-resistant isolates of *A. alternata* (O'Neill & Griffin, 1991).

Lupin

The fungal disease anthracnose can cause extensive necrosis and growth distortion in lupins. The causal pathogen is one or more *Colletotrichum* species. The disease has been attributed to both *C. gloeosporioides* and *C. acutatum,* whilst more recently the name *Colletotrichum lupini* was proposed for the pathogen. Because the disease also affects field lupin grown as an oil or fodder crop there has been extensive research into the control of the problem, although the disease was also examined on ornamental lupin by Maude (1994) – see *Lavatera,* above.

Thomas & Adcock (2004) found that dry heat for 4-7days at 15°C, or up to 4 days at 70°C will significantly reduce and possibly eliminate anthracnose infection in lupin seed. Temperatures below 70°C had little or no effect on germination of *L. angustifolius* seed.

Santen *et al.* (2004) investigated non-chemical methods to control anthracnose on white lupin (*Lupinus albus)* in organic farming systems in Germany. Hot water treatment (no temperatures or duration given in abstract) was very effective, but thought to be unfeasible for treating large batches of seed. Seed storage for a period of two years reduced the level of infected seed from 67% to less than 1% without affecting germination. Electron radiation treatment and some plant extracts (detail not provided in abstract) also reduced disease levels.

Rutskaya & Sviridenko (2005) tested over 180 chemicals, alone or in combination, as seed treatments. Around 30 were effective (not all listed in abstract). Tests on *Lupinus angustifolius* and *L. albus* showed that carbendazim, carboxin + thiabendazole, and tolylfluanid were effective for seed treatment at 1.5 kg/t. Note that approval for use of tolylfluanid-based products in the UK was withdrawn in 2007.

Thomas & Sweetingham (1999) conducted a number of field trials on seed treatments against anthracnose, from which they concluded that thiram (100 g a.i./100 kg seed) and carbendazim (50 g a.i./100 kg seed) gave good reductions in disease levels, but poor control of brown spot caused by *Pleiochaeta setosa.* By contrast, iprodione (25 g a.i./100 kg seed) gave good control of brown spot but poor control of anthracnose. Carbendazim seed treatment has occasionally reduced lupin emergence. Carbendazim has a temporary Annex 1 listing only following the EU review programme (91/414/EU) and uses of the fungicide are due to be revoked on 30 June 2008.

Foliar application of azoxystrobin, chlorothalonil and mancozeb during flowering reduced but did not eradicate seed infection (Thomas *et al.,* 2007).

Marigolds

Wu *et al.* (2001) found that seed treatment with iprodione at 200 ppm significantly increased the emergence of pot marigold (C*alendula officinalis*) seed contaminated with *Stemphylium vesicarium*, compared to an untreated control. They also found that treating seed of African marigold (*Tagetes erecta*) with mancozeb against *Alternaria tagetica* (a leaf spot pathogen not present in the UK) was highly effective, whilst the biocontrol agent *Bacillus azotoformanis* (applied at 1 x 10^9 cfu/mL) was also effective.

Pyrethrum

Pethybridge *et al.* (2006) examined seed-borne infection of *Pyrethrum* with *Phoma ligulicola* (the cause of the disease called ray blight which also affects chrysanthemum). Seed treatments with fludioxonil or thiabendazole + thiram significantly reduced the incidence of seed-borne *P. ligulicola* and increased seed germination and seedling survival.

Zinnia

The fungal pathogen *Alternaria zinniae* can cause damping-off and severe leaf and flower necrosis and is commonly seed-borne. Recommended seed treatments include mancozeb, thiram, steam/air treatment for 30 minutes at 60°C and hot water treatment for 30 minutes at

52°C (followed by cold water) (Beaumont *et al.,* 1958). Thiram dust treatment gave excellent control without reducing seed germination.

Franklin & Goodwin (1982) found that hot water treatment of zinnia seeds sufficient to kill *Alternaria zinniae* (30 minutes at about 55°C) resulted in large reductions in seed germination. In an attempt to overcome seed-imbibition and leaching of solutes associated with reduced germination, treatment in hot concentrated salt solutions was evaluated. By use of calcium chloride at 1.5M, infection levels were kept below 5% and germination above 50%.

Various ornamentals

An experimental aerated steam treatment machine was developed at the Institute for Horticultural Development in Australia and evaluated against a range of seed-borne diseases of ornamentals (Mebalds *et al.,* 1996). Results were:

- Stocks – seed treatment at 54°C for 30 minutes gave 100% kill of seed-borne *Alternaria alternata, Fusarium oxysporum* and *Xanthomonas campestris*. Seed germination was 91% before treatment and 90% after.
- Lobelia – treatment at 50°C for 20 minutes gave 98% kill of *Alternaria alternata*. Germination was 81% before treatment, 75% after.
- Cineraria – treatment at 54°C for 30 minutes gave 100% kill of *Alternaria cinerariae*. Germination was 91% before treatment, 94% after.
- Phlox – treatment at 49°C for 20 minutes gave 100% kill of *Stemphylium botryosum*. Germination was 72% before treatment, 71% after.

Recent work on other crops

Celery: Evaluation of alternative seed treatments for the control of Septoria apiicola (celery leaf spot) - HDC Project FV 237a

This project ran initially from 2002 to 2003, with a further one year extension completed in 2007. It compared the industry standard treatment of a warm water thiram seed soak with a range of other seed treatments, including hot water treatment, disinfectants, UV light, microwaves, fungicides, essential oils and biological controls.

Essential oils (pine oil, eucalyptus oil and winter savory oil) and the biological control *Pythium oligandrum* (as the product 'Polyversum') were ineffective. UV treatments also had negligible effects as pigments in the seed absorbed them. Microwaves reduced spore germination but also affected seed vigour.

Hot water treatment (10 minutes at 48ºC), the disinfectant Jet 5 (peroxyacetic acid - 1 hour soak in a 20% solution) and the fungicide seed treatment Wakil XL (cymoxanil + metalaxyl-M + fludioxonil) gave significant reductions in the levels of *Septoria apiicola* without affecting seed vigour. However, the industry standard thiram soak was the only treatment that completely eliminated *S. apiicola* from the seed. It should be noted that Jet 5 cannot currently be developed as a commercial seed treatment, since under current legislation peroxyacetic acid is approved in the UK for use on flower bulbs and potato tubers, and for disinfection of glasshouses, warehouses and agricultural tools and equipment only.

Bulb onions: Evaluation of alternative seed treatments for the control of neck rot (Botrytis allii) - HDC Project FV 263

This project ran from 2004 to 2006. The industry standard seed treatment of Hy-TL fungicide (thiabendazole + thiram) was compared to a range of other treatments, including hot water, disinfectants and other fungicides.

The fungicide Raxil (tebuconazole), whilst eliminating the pathogen, reduced seed germination dramatically. Wakil XL (cymoxanil + metalaxyl-M + fludioxonil) was effective against external botrytis but less effective against internal contamination. An experimental fungicide (unnamed due to confidentiality agreement) was effective against both external and internal botrytis.

Hot water treatment at 45ºC for 30 or 45 minutes, following a pre-soak at 20ºC for 18 hours, reduced *B. allii* infection dramatically with no adverse effects on germination.

Of the disinfectants, Jet 5 (6 hour soak in a 2% solution, or 20 minute soak in a 10% solution) provided the most consistent control of *B. allii*, with no adverse effects on germination.

Seed Treatments for Organic Vegetable Production (STOVE)

This EC co-funded project ran from 2003 to 2006, and examined a range of alternatives to chemical seed treatments for use in organic vegetable production. A number of host / pathogen combinations were used in the research:

- Carrot: *Alternaría* spp., *Xanthomonas campestris*
- Brassicas: *Alternaria brassicicola, Xanthomonas campestris*
- Parsley: *Septoria petroselini*
- *Phaseolus* bean: *Colletotrichum lindemuthianum*
- Pea: *Ascochyta pisi*
- Lamb's lettuce: *Phoma valerianellae*

Treatments evaluated included physical methods, plant extracts, micro-organisms and resistance inducers. Information on the results of the research can be found at www.stove-project.net . A brief summary of the main results is given below:

Physical treatment

The three treatments evaluated were hot water, aerated steam and electron treatment.

i. Hot water

The optimum range for temperature and duration varied with the crop / pathogen combination, but in all cases was between 50°C to 53°C for 10 to 30 minutes (Nega *et al.,* 2003). In most cases efficacy of hot water treatments against *Alternaria* species was high (>95%). Efficacy against *Phoma* species was also very good (80-95%). For *Xanthomonas campestris*, good results were obtained at 50°C for 30 minutes.

ii. Aerated steam

The technique used was a system known as Thermoseed®, developed in Sweden by Acanova for cereals, and now being used commercially in that country by Svenska Lantmännen (SvL). First used in 2006, the equipment can treat up to 200 tonnes of cereal seed a day. Due to the inherent variation in sensitivity to treatment between seed lots (see below), representative samples from each seed lot are laboratory-tested and the precise requirements for the lot determined from mathematical models prior to Thermoseed® treatment. See www.thermoseed.com for more details.

Tests on the various crop / pathogen combinations in the STOVE project showed that Thermoseed® reached high disinfestation rates against both fungal and bacterial pathogens without affecting seed germination. It was concluded that the technique has 'high potential for practical use in horticultural crops in the near future'.

iii. Electron treatment

This technique utilises the biocidal effect of low-energy electrons. The effect can be controlled by adjusting the depth of penetration of electrons so that only the seed coat is treated (there are concerns that the DNA of the seed could be damaged if the electrons penetrate the embryo). The technology is currently utilised in Germany as the e-ventus® stationary and mobile seed treatment units (Fraunhofer Institute and Schmidt-Seger AG – see http://www.fep.fraunhofer.de/enu/versanl/e-ventus.asp for more details) for cereals, with throughputs of up to 30 tonnes/hour. This technique was also effective against many of the host / pathogen combinations in the STOVE project.

In general, both the hot water and aerated steam treatments were more effective than the electron treatment. Hot water and aerated steam had similar disinfection efficiencies, with the main advantage of aerated steam being the elimination of the need for post-treatment drying (S. Roberts, pers. comm.).

iv. Variation between species / seed lots

As in much of other research into seed treatment, variations were found in sensitivity to all of the physical treatments between species, cultivars of the same species, and lots/batches of the same cultivar. Chlorophyll fluorescence studies showed that in general, more mature seeds were less prone to damage from hot water and aerated steam treatments than less mature seeds. Maturity was less of a problem with the electron treatment.

Plant extracts, micro-organisms & resistance inducers.

Results were more variable, with the performance of micro-organisms and plant extracts often better in glasshouse trials than in the field. Most resistance-inducing compounds were excluded after the first round of screening.

Thyme oil treatment showed promise against a number of the host / pathogen combinations, and a number of micro-organisms showed promise against specific pathogens.

Neither Thermoseed nor e-ventus treatment systems are currently available in the UK, and there may be a potential problem with cost of treatment, particularly for the Thermoseed, if

each individual seed lot has to go through a laboratory calibration process before treatment – a treatment economic for large batches of cereal seed may not be for small batches of ornamentals.

Other work on fungal pathogens

Du Toit and Derie (2005) evaluated a range of fungicide seed treatments for the control of *Phoma lingam* ('black leg' or canker) of cauliflower. Benomyl, boscalid, thiabendazole, pyraclostrobin + boscalid and iprodione gave complete control of the disease on seed carrying 4% infection. Fludioxonil and azoxystrobin also gave good control, whilst thiram and thiophanate-methyl were slightly less effective. There was little or no effect on germination of any of the treatments.

Chand *et al.,* (2005) examined a range of biological control agents (BCAs) and plant extracts as seed treatments against fusarium wilt of chickpea (*Fusarium oxysporum* f. sp. *ciceri*) (a number of ornamental plants are also affected by specific fusarium wilt diseases). Treatment with the BCA *Trichoderma viride* was found to be partially effective, giving 77.8% control. The best plant extract was garlic (*Allium sativum*), which reduced disease incidence from 65.9% in the untreated control to 23.6%.

Burgess *et al.* (1997) evaluated an isolate of the BCA *Gliocladium roseum* against *Botrytis cinerea* on chickpea seed. The isolate proved highly antagonistic to the pathogen, with seed treatment at 3 x 10^8 conidia/mL increasing establishment from 1.4% to 69.4%, an increase equivalent to that resulting from thiram seed treatment.

Other work on bacterial pathogens

Thermotherapy (most commonly hot water, but also aerated steam and dry heat) treatments are used most frequently to control seed contamination by bacterial pathogens such as *Xanthomonas campestris* and *Pseudomonas syringae*. Such treatments have been reviewed by Maude (1996). *Xanthomonas campestris* was also one of the pathogens studied in the STOVE project.

Most of the fungicides used to treat seed against fungal diseases have little or no effect on bacterial pathogens, and there has thus been little work on the use of chemical seed treatments for control of bacterial diseases. Copper and zinc do have activity against bacteria, however, and some workers (e.g. Schaad *et al.*, 1980) have attempted to combine hot water treatment with the use of one of these metals to improve control.

Antibiotic seed soaks have been evaluated against various bacterial pathogens in a number of countries, but use of antibiotics to control plant diseases is not permitted in the EU.

McMillan (1987) compared hot water treatment with seed soaks in formaldehyde or sodium hypochlorite for the control of bacterial spot of tomato caused by *Xanthomonas campestris* pv. *vesicatoria*. All treatments gave 100% control when evaluated *in vitro*, with no adverse effects on seed germination. Results were more variable when used on grower's holdings, and were largely dependent on the adequacy of the grower's facilities for maintaining accurate temperature control for the hot water treatment, or for the flushing of applied sodium hypochlorite from the treated seed. Formaldehyde has no potential as a seed treatment in the UK as it is not being supported for EU Annex 1 listing as a pesticide.

Brown *et al.* (2001) examined the use of low dose UV-C treatments of cabbage seed to induce host resistance (hormosis) against black rot (*Xanthomonas campestris* pv. *campestris*). The optimum UV-C dose of 3.6 kJ^{-2} was effective in reducing black rot and the population density of *X. campestris* pv. *campestris* in infected cabbage leaves.

Work on viruses

A number of virus diseases of ornamentals are known to be seed-transmitted, and were listed in the June 2007 annual report for this project. However, there is little or no information as to how common this infection route is in commercial crops of ornamental plants. Seed transmission is, in practice, likely to be a minor source of infection in comparison to the transmission of viruses to the growing crop by vectors (aphids, thrips, whiteflies etc.), and the spread of viruses in infected cutting material produced from contaminated mother plants.

Many of the seed-borne viruses occur as deep-seated infections within the embryo, as the infection is derived systemically from the parent plant. Attempts to control these viruses with thermotherapy or chemical treatments are usually not completely successful, as treatments that inactivate the virus generally also impair seed viability (Maude, 1996).

Where the infection is less deep-seated, in the endosperm or particularly on the seed coat, treatment may be more successful. Much of the work on seed treatments against viruses transmitted in this way has been carried out on highly contagious viruses of edible crops, e.g. tomato mosaic virus (ToMV) and pepino mosaic virus (PepMV) on tomatoes. A range of treatments can be used (Fletcher, 1983, Jones, 2005), including acid extraction (with concentrated hydrochloric acid), seed soaks in sodium hypochlorite or trisodium phosphate, or dry heat treatment if the virus-infection is more deep-seated (although once again the

temperatures required (70°C or 80°C) may affect germination). Currently it is a statutory requirement for commercial plant propagators in the UK to treat tomato seed with sodium hypochlorite for control of PepMV.

Current fungicide seed treatments

Iprodione (e.g. Rovral WP) is currently the only fungicide with a label recommendation for the treatment of seeds of ornamental plants. Seed can be soaked for 8 hours in a suspension of 1 g of Rovral WP in 1 litre of water. The label states that lobelia, nemesia, wallflower, zinnia and cineraria species may be safely treated. The specific recommendation is for control of *Alternaria* species. Iprodione, when applied as a spray, also has activity against sensitive strains of *Botrytis*, and some activity against *Rhizoctonia*, but it is unclear how effective the product is against seed-borne *Botrytis*. There are also problems with fungicide resistance to iprodione, both in *Botrytis cinerea* (Anon, 2006) and *Alternaria alternata* (O'Neill & Griffin, 1991).

A large number of seed treatment products are available for cereals or other arable crops such as peas, beans, linseed and oilseed rape (Whitehead, 2007). It is quite possible that some of these fungicides could also have activity against some of the major seed-borne fungal diseases of ornamentals such as *Alternaria, Botrytis* and *Colletotrichum*, though safety to seeds and seedlings is a major consideration here and would require validation. In addition to activity against seed-borne pathogens, seed treatments containing thiram or metalaxyl-M may also give some protection to the germinating seed and seedlings against damping-off and root rot pathogens such as *Pythium* and *Phytophthora*, which can be present as contaminants of soil, growing media, pots, trays, seeding apparatus and other equipment.

Suitability of different treatments for experimental evaluation against seed-borne diseases of ornamentals

This review has examined work on a large number of host / pathogen combinations for the treatment of seed-borne disease. It is unsurprising, therefore, that no single treatment has been found to be successful in all cases. However, there are clear indications from the work that certain treatments have greater potential than others. The relative merits of the various treatments, and those worthy of further consideration, are as follows:

Fungicides

A clear advantage in the evaluation of fungicides for their efficacy in controlling seed-borne diseases is that the product can be easily applied to seed of a range of different ornamental

species, enabling initial screening against a range of host / pathogen combinations in a relatively short timescale. However, adjustments in the rate of application may be necessary, dependant on the effects of the treatment on the pathogen and the seed itself.

Of the fungicides currently available for the treatment of seed-borne diseases of other crops, the following are amongst those that would merit evaluation against seed-borne pathogens of ornamentals:

Wakil XL (cymoxanil + metalaxyl-M + fludioxonil). This product gave significant control of *Septoria apiicola* on celery seed (Green, 2002) and external *Botrytis allii* on onion seed (Green, 2006). Fludioxonil was also effective against *Phoma ligulicola* on pyrethrum (Pethybridge *et al.*, 2006).

Hy-TL (thiabendazole + thiram). This is the industry standard for control of *Botrytis allii* on onion seed (Green, 2006). The active ingredients, either in combination or as single products, have also been effective as seed treatments against a range of pathogens of ornamentals, such as *Colletotrichum* on lupin and lavatera (Maude, 1994; Thomas & Sweetingham, 1999) and *Phoma ligulicola* on pyrethrum (Pethybridge *et al.*, 2006). Hy-TL is offered by commercial seed treatment companies as a treatment against *Colletotrichum* on field lupins, and is stated to be effective where infection is below 15%. Treatment with Hy-TL is recommended by PGRO where there is a risk of seed-borne infection by *Colletotrichum*. The thiram constituent also gives some protection against damping-off fungi.

Agrichem Hy-Pro Duet (prochloraz + thiram). Thiram has been shown to be effective against a range of seed-borne pathogens (Beaumont *et al.,* 1958; Green, 2002; Thomas & Sweetingham, 1999), whilst prochloraz (as the commercial product Octave) is used routinely against both *Botrytis* and a wide range of foliar pathogens of ornamental plants. Seed treatment products combining prochloraz with more modern triazole fungicides, e.g. Kinto (prochloraz + triticonazole) and Galmano Plus (prochloraz + fluquinconazole) might also merit attention.

Iprodione (against *Alternaria* species and *Botrytis*) and Thiram as a straight product (against a range of pathogens) should also be included, as these products can be used as seed soaks by growers without the need for more expensive seed treatment equipment.

Redigo Twin (fluoxastrobin + prothioconazole). Strobilurin fungicides are known to have good protectant properties against fungi from a large number of different groups. The

strobilurin fungicide fluoxastrobin is available in combination with the triazole fungicide prothioconazole as Redigo Twin seed treatment for cereals.

An alternative to seed treatments that can be readily used by growers is the application of fungicides as sprays to the newly emerged seedlings or young plants. It would therefore be worth comparing the efficacy of seed treatments versus foliar sprays (e.g. of prochloraz or thiophanate-methyl) in trials. Ideally, to avoid the use of unnecessary fungicide sprays by growers, batches of seed should to be tested by a laboratory to determine which stocks were contaminated by a seed-borne pathogen, and require treatment.

Thermotherapy

Of the thermotherapy treatments described in this review it is hot water treatment which would lend itself most readily to application on grower's holdings. However, there will very probably be a different optimum treatment temperature and duration for each host / pathogen combination which, given the large number of different species of ornamentals grown, would require a great deal of experimental work. A suitable compromise would be to select two or three temperature and timing combinations and test these against the selected range of seed-borne pathogens targeted in this project.

The advantages of aerated steam treatment over hot water treatment have already been discussed. However, at present it is unlikely that this treatment would be suitable for application by growers themselves. The Thermoseed® treatment evaluated in the STOVE project is currently unavailable in the UK. Even if it became available to UK growers it is possible that the cost could be excessive for seed treatment of ornamentals, given that seed lots of ornamentals are generally quite small, and that each would have to go through the laboratory calibration process prior to Thermoseed® treatment.

Electron treatment

The e-ventus electron treatment system is also not yet available in the UK. Evaluation of this system would only be worthwhile if there is a strong chance that it will become available for use by UK growers in the near future.

Disinfectants

Whilst commercial disinfectants cannot be used for seed treatment unless they are specifically approved for this purpose, it may be worth evaluating a product such as Jet 5 in this project, as it has shown promise against other seed-borne pathogens (Green, 2002; 2006) and can be tested quite easily against a range of seed-borne diseases of ornamentals.

Approval could be sought if the treatment shows promise, and it is likely that growers could use such a treatment on the nursery.

Plant extracts

As thyme oil gave promising results against a number of host / pathogen combinations on vegetable crops in the STOVE project, it could be worth evaluating its efficacy against seed-borne pathogens of ornamentals; it is noted however that plant extract oils can be expensive.

Biological Control Agents (BCAs)

It has been difficult to identify a single promising BCA from the research reviewed. This is unsurprising, given the complexity of the interactions that occur when a biological control agent comes into contact with its potential target. In addition to the interaction between the BCA and the target, numerous other factors (e.g. temperature, humidity, and the presence of other fungi, bacteria, etc. on the seed or other treated surface) can affect the outcome. For this reason, many promising BCAs identified in laboratory tests (where conditions can be controlled more precisely) fail to perform satisfactorily when evaluated *in vivo*.

Given the number of different seed-borne pathogens found on ornamental plants it would also be very surprising if any one BCA was universally effective. However, it is possible that a given BCA might prove to be effective against a particular host / pathogen combination, and for this reason it would be unwise to rule out biological control. Even though they are currently unapproved in the UK, the BCAs that are already formulated as commercial products with recommendations for seed treatment (Mycostop and Polyversum) would be most suitable for evaluation. It may also be worth testing *Gliocladium roseum* against *Botrytis cinerea*, given the results obtained by Burgess *et al.,* 1997.

UV treatment

This is still at an early stage of development as a seed treatment. Whilst UV-C treatment showed some promise against *Xanthomonas campestris* pv. *campestris* on cabbage (Brown *et al*., 2001), both this and other UV treatments were ineffective against *Septoria apiicola* on celery due to absorption of the UV by pigments in the seed coat (Green, 2002). Due to the volume of work likely to be required to develop UV seed treatment for ornamentals, it is recommended that this area of treatment be given lower priority than others. If further work is to be carried out, Green (2002) recommends that the following preliminary steps be undertaken:

- The inherent UV sensitivity of the target pathogen should be assessed *in vitro* before seed treatments are attempted.

- The transmission spectrum of the solution produced by soaking seeds should be quantified spectrophometrically. This would help to identify systems, such as celery, where compounds leached from the seed greatly attenuate the UV reaching the seed.

Microwave treatment

Microwaves have been used to eradicate both surface-borne and more deep-seated infection on seeds and planting material of a diverse range of crops. The duration and power of the treatment will vary between plant species. The effect of microwave treatment on seed germination can also vary according to both seed size and seed moisture content. Adverse effects are reduced when smaller seeds or those with lower moisture content are treated (Hankin & Sands, 1977; Jolicoeur, 1982). One possible explanation for the adverse effects on larger seeds is that these are unable to radiate heat away from the seed during treatment.

Whilst microwave treatment had adverse effects on seed germination of celery and variable efficacy against *Septoria apiicola* (Green, 2002), the use of microwaves on a range of other crops has given good results (against both surface-borne and more deep-seated infection). For example, microwave treatment was used to eradicate seed-borne pathogens (including *Fusarium* spp., *Cladosporium, Colletotrichum, Diplodia* and *Xanthomonas campestris* pv. *manihotis*) in cassava true seed (Lozano *et al*, 1986). The efficacy of microwave treatment against seed-borne pathogens is relatively straightforward to evaluate and this, coupled with the fact that the treatment could be undertaken by growers, makes it worthy of consideration.

Conclusions

- Out of 25 commercial seed lots of 15 ornamental species for which tests were completed in year 2 of this project, the suspect pathogens recovered were *Alternaria* species (on coreopsis, cineraria, lobelia, senecio, tagetes and zinnia), *Botrytis cinerea* on zinnia, *Fusarium oxysporum* on cyclamen, *Phoma* sp. on lychnis, and bacteria on alyssum, antirrhinum, geranium and salvia.
- None of the pathogenicity tests done using suspect pathogens isolated from seed in year 2 were positive, although tests are still ongoing for some crop / pathogen combinations.
- Attempts to locate large batches of seed (sufficient for experimental seed treatments) that were known to be naturally infested by one or more of the above pathogens were unsuccessful. Work on this objective will continue in year 3.
- From a literature review, the following options for treatment of ornamental seed were highlighted as high or medium priority for further work: fungicide seed treatments either by seed companies or as soak treatments by growers, seedling sprays with fungicides, hot water treatment, aerated steam treatment, essential oils and microwave treatment.

Technology transfer

Project review meeting, Stratford on Avon, 27 May 2008

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