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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.

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GROWER SUMMARY

Headline

- A review of diseases of 36 protected ornamental crops identified 246 pathogens as seed-borne.
- The majority of the 86 commercial seed lots of 24 ornamental plant species tested over three years were found to be free of fungi and bacteria that might cause disease in these species.
- The pathogens detected most frequently were *Alternaria* species and *Botrytis cinerea*.

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 were 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

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.

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Summary of the project and main conclusions

Seed-borne diseases of protected bedding and pot plant species

A review of worldwide literature was undertaken to identify seed-borne diseases of common bedding and pot plant species grown in the UK. Lists of confirmed and suspected seed-borne diseases were compiled, covering bacterial, fungal, virus and viroid diseases. In total, 246 pathogens affecting 36 hosts are listed as confirmed seed-borne diseases. The majority (68%) are fungal pathogens; *Botrytis cinerea* and various species of *Alternaria* have been recorded on several hosts. Some common diseases (e.g. *Pythium* root rot; downy and powdery mildews) are known to be seed-borne on only a few hosts. The possible occurrence of downy mildew on bedding plant seed e.g. pansy and *Impatiens* is being investigated in a separate project (PC 230). The full lists are available in the Science Section of the June 2007 annual report; a condensed list is shown in Table 1.

Table 1: List of confirmed fungal and bacterial seed-borne diseases of important bedding and pot plant species grown in the UK

Ornamental species and associated pathogen(s)	Disease symptom	Pathogen reported in the UK*
Alyssum		
Alternaria sp.	Leaf spot	Yes
Stemphylium botryosum	Leaf spot	Yes
Sclerotinia sclerotiorum	Tissue rot	Yes
Antirrhinum majus (snapdragon)		
Alternaria alternata	Seedling malformation	Yes
Alternaria spp.	-	-
Botrytis cinerea	Grey mould	Yes
Colletotrichum antirrhini	Anthracnose	-
Fusarium spp.	-	-
Heteropatella antirrhini	Leaf spot	-
Phyllosticta antirrhini	Leaf spot and stem rot	Yes
Pleospora herbarum	-	Yes
Pseudomonas syringae pv. *antirrhini*	Bacterial leaf spot	Yes
Puccinia antirrhini	Rust	Yes
Cheiranthus spp. (wallflower)		
Alternaria brassicicola	Black leaf spot	Yes
Alternaria cheiranthi	Black mould	-
Ascochyta cheiranthi	Leaf and stem rot	-
Phoma spp.	-	-
Sclerotinia sclerotiorum	-	Yes
Xanthomonas campestris pv. campestris	Bacterial wilt	Yes
Cyclamen persicum (cyclamen)		
Botrytis cinerea	Grey mould	Yes
Colletotrichum gloeosporioides	Anthracnose	Yes

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Table 1 continued:

Ornamental species and associated pathogen(s)	Disease symptom	Pathogen reported in the UK*
Fusarium oxysporum f. sp. c*yclaminis*	Wilt	Yes
Ramularia cyclaminicola	Leaf spot/stunt	-
Septoria cyclaminis	Leaf spot	-
Impatiens spp. (Busy Lizzie or snapweed)		
Alternaria zinniae	-	Yes
Phyllosticta impatientis	-	-
Plasmopara obducens	Downy mildew	Yes
Rhizoctonia solani	-	-
Lobelia		
Alternaria alternata	Leaf spot and stem rot	Yes
Lupinus spp. (lupin)		
Botrytis cinerea	Grey mould	Yes
Colletotrichum acutatum	Anthracnose	Yes
Diaporthe woodii	Phomopsis stem blight	-
Erwinia sp.	-	-
Fusarium oxysporum	Wilt	-
Gibberella avenacea	-	-
Glomerella cingulata	Anthracnose	Yes
Pseudomonas spp.	-	-
Sclerotinia sclerotiorum	-	Yes
Stemphylium sp.	Leaf spot	-
Verticillium albo-atrum	Wilt	Yes
Verticillium sp.	Wilt	-
Nicotiana spp. (tobacco)		
Alternaria alternata	-	-
Alternaria longipes	-	-
Alternaria zinniae	-	Yes
Botrytis cinerea	Grey mould	Yes
Cercospora nicotianae	Green spot	-
Colletotrichum tabacum	Anthracnose	-
Corynebacterium fascians	-	-
Erwinia carotovora ssp. or pv *carotovora*	Tobacco hollow stalk	-
Erwinia spp.	-	-
Peronospora tabacina	Downy mildew	Yes
Pseudomonas aeruginosa	Phillipine leaf spot	-
Pseudomonas syringae pv. *Mellea*	Wisconsin leaf spot	-
Pseudomonas syringae pv. *Tabaci*	Wildfire	-
Xanthomonas heterocea	Bacterial leaf spot	-
Pelargonium sp. (pelargonium, geranium)		
Pseudomonas sp.	Bacterial leaf spot	Yes
Phlox drummondii (phlox)		
Alternaria sp.	-	-
Cochliobolus lunatus	-	-
Rhizoctonia solani	Damping off	-
Septoria drummondii	Leaf spot	Yes
Primula spp. (primrose, polyanthus)		
Botrytis cinerea	Grey mould	Yes
Phyllosticta primulicola	Leaf spot	-
Pseudomonas syringae pv. *primulicola*	Bacterial leaf spot	-
Ramularia agrestis	Leaf spot	Yes

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Table 1 continued:

Ornamental species and associated pathogen(s)	Disease symptom	Pathogen reported in the UK*
Senecio cruentus (cineraria)		
Alternaria cinerariae	Leaf spot	Yes
Botrytis cinerea	Grey mould	Yes
Erysiphe cichoracearum	Powdery mildew	Yes
Viola (pansy, violet)		
Colletotrichum violae-tricoloris	Anthracnose	-
Mycocentrospora acerina	Halo blight	Yes
Phoma sp.	-	-
Ramularia lactea	Leaf spot	Yes
Rhizoctonia solani	-	Yes
Sphaceloma violae	Scab	-
Urocystis violae	Smut	-
Zinnia elegans (zinnia)		
Alternaria zinniae	Leaf spot/blight	Yes
Botrytis cinerea	Grey mould	Yes
Colletotrichum acutatum	-	-
Erysiphe cichoracearum	Powdery mildew	-
Glomerella cingulata	-	-
Phyllosticta sp.	-	-
Rhizoctonia solani	Damping off	-
Xanthomonas campestris pv. *zinniae*	-	-

More detailed crop/pathogen lists are provided in the Science Section of the 2007 Annual Report.

*****Pathogen recorded in the UK but not necessarily on the listed host. The frequency of a disease occurrence does not necessarily reflect current occurrence on seed due to the possibility of various other sources of outbreaks.

Seed-borne pathogens and seed transmission

Seed-borne plant pathogens may occur as:

- *Contamination* – the pathogen is carried as trash with the seed but is not attached to it (e.g. sclerotia of *Sclerotinia sclerotiorum).*
- *Superficial inoculum* – the pathogen is located on the outside of the seed or fruit coat.
- *Internal inoculum* – the pathogen is located within the seed, either in seed or fruit coat tissues, in storage tissues (e.g. endosperm or cotyledon) or deep-seated in the embryo.

The presence of a plant pathogen on (or in) seed does not necessarily mean that an infected seedling will result. A disease is only said to be seed-transmitted once it has been demonstrated that a seed-borne pathogen can give rise to infected seedlings. There is a potential risk that all the types of seed-borne infection listed above may give rise to infected seedlings (i.e. seed transmission may occur). However, the risk of seed transmission is greater where there is internal inoculum and the pathogen grows systemically with the plant.

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Any resultant external disease symptoms occurring as a result of this systemic infection will also be dependant on the prevailing weather and cultural conditions at the time.

It should be noted that not all strains of a particular fungus will necessarily cause disease. Hence in this project, where fungi recovered from seed have been identified as belonging to a plant pathogenic species or genus, they are referred to as 'suspected' plant pathogens. Pathogenicity tests were conducted on a number of isolates in order to provide more definitive information (see below).

Prevalence of seed-borne pathogens

Standard methods were used to test commercial seed lots of ornamental plant species for key seed-borne fungal and bacterial pathogens. Surface disinfection of seed was used to distinguish superficial from more deep-seated inoculum. Fungal isolates were identified to genus or species level according to morphological features and the appearance of colonies in culture; bacterial isolates were identified by the Food and Environment Research Agency (Fera). Examination of seed transmission by growing-on tests was not done in this project. Seeds were supplied in sealed packets from two seedhouses in years 1 and 2 and from eight seedhouses in year 3. Key results of the seed tests are given below.

From a total of 86 seed lots of 24 ornamental species tested, 55% were found to be free of fungi that might cause disease in these species. The suspected plant pathogens found most frequently were *Alternaria* species (24% of seed lots on 10 ornamental species) and *Botrytis cinerea* (17% of seed lots on 11 ornamental species). Other suspected plant pathogens detected were *Fusarium* spp. (3% of seed lots, affecting *Cyclamen* and stock), *Pythium* spp. (on one lot each of *Aquilegia* and stock) and *Phoma* spp. (on 2 lots of *Lychnis*). Bacteria were recovered from 9% of seed lots, affecting 6 ornamental species, but none were identified as species of *Pseudomonas* or *Xanthomonas*, the two bacterial genera that most commonly cause bacterial diseases on seed-raised ornamentals. A total of 11 batches of lupin seed were tested and no *Colletotrichum acutatum* was found. No *Sclerotinia sclerotiorum* was found associated with or grew from seed of any species examined and tested.

Pathogenicity tests

Isolates of selected fungi and bacteria recovered from seeds were tested for their ability to cause disease in plants of the species from which they were obtained. The isolates used

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were ones identified as suspected fungal or bacterial pathogens; where available, they were taken from surface disinfected seed in order to minimise the chance of selecting saprophytic isolates. Isolates were sub-cultured to check for purity and then grown on agar for production of bacterial cells, fungal spores or fungal mycelium for use in pathogenicity tests.

Bacteria were generally tested by stab-inoculation of a cell suspension into stems of the test plant. Fungi were tested by spray-inoculation with a spore suspension or by placing mycelial plugs on agar onto leaves or stems of the test plant. One exception was *Fusarium oxysporum* recovered from *Cyclamen* seed, which was tested by soaking roots of young plants in a spore suspension of the fungus. Control plants were inoculated with sterile distilled water or an agar plug. Plants were enclosed in a polythene bag for at least 48 h after inoculation to create a high humidity. Plants were then incubated in the laboratory or glasshouse for 2-4 weeks and examined for symptom development.

Bacteria obtained from seeds of *Alyssum*, *Aquilegia*, *Cineraria*, *Pelargonium*, salvia, *Tagetes*, *Verbena* and wallflower caused no symptoms when inoculated into the respective species. A slight stem rot occurred on *Antirrhinum* following inoculation with an unidentified bacteria; however, subsequent tests on the isolate used did not identify any known plant pathogenic bacteria.

The results of these pathogenicity tests on bacteria were consistent with the results of identification tests. None of a range of bacterial cultures submitted to Fera was identified as plant pathogenic *Pseudomonas* or *Xanthomonas* species; many of the cultures recovered from seeds were identified as species of *Bacillus* and *Brevibacillus* species considered as secondary saprophytic species or 'opportunists' colonising previously damaged tissues.

It is worth noting that whilst many bacteria can be seed-borne the infection rate may be very low e.g. <1:10,000 seed (<0.01%). In this scenario, the chance of detection using conventional culture plate isolation techniques is extremely low. Alternative methods, including modern molecular techniques, provide greater promise though the economics of such independent testing on relatively small batches of ornamental seed may continue to be prohibitive for some time.

Isolates of *Alternaria* spp. obtained from *Cineraria*, *Lobelia*, phlox, *Tagetes* and *Zinnia* caused limited or spreading lesions on their respective hosts. One *Alternaria* sp. isolate from *Tagetes* caused a black rot of flower buds. Seed-borne *Alternaria* spp. have been previously reported in all of these crop species. The symptom observed on inoculated plants was

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usually an irregularly shaped lesion and not the zonate spotting typical of many *Alternaria* diseases; possibly this difference was a result of the inoculation method used.

Isolates of *Botrytis cinerea* obtained from seed of *Primula* and *Zinnia*, and of *Botrytis streptothrix* from *Lobelia*, caused rotting when inoculated onto undamaged tissue of their respective hosts. These results confirm that seed-borne isolates of *B. cinerea* are capable of causing disease in *Primula* and *Zinnia*.

Two isolates of *Fusarium oxysporum* recovered from seed of *Cyclamen* caused vascular browning in the corms of *Cyclamen* at 12 weeks after root-dip inoculation. This symptom is typical of *Fusarium* wilt in *Cyclamen*. *F. oxysporum* was recovered from the stained vascular tissue in the corms.

Other fungi that caused disease symptoms in our inoculation tests were *Fusarium* sp. on *Lobelia* and *Phoma* sp. on *Lychnis*.

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 were reviewed in Year 2. Seed and seedling treatments that warrant evaluation on ornamentals are summarised in Table 2. Within this project, the effects of microwave treatment, aerated steam treatment and one fungicide seed treatment on seed-borne fungi were examined.

Table 2: 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
	Rovral Aquaflo	Iprodione	*Alternaria, Botrytis*	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

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Table 2 continued:

Treatment method (and potential provider)	Example product	Active substances in product	Example target	Priority
Hot water seed treatment (by grower)	-	-	*Alternaria, Botrytis,* bacteria	High
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.

Fungicide seed treatment

Seed treatment with Rovral AquaFlo (iprodione) was examined for the control of *Alternaria* sp. on a batch of *Lobelia* seed naturally infected with the fungus. Efficacy of a 5 minute dip of 1% sodium hypochlorite solution was also examined. Both treatments appeared to reduce levels of *Alternaria* from 1% to around 0.3%, but differences were not significant. Rovral AquaFlo seed treatment had no effect on seed germination. In 2009, Rovral AquaFlo was granted an on-label approval for the treatment of ornamental seeds, as a dip at 0.1 litre product/100 litres water. Further work is required to confirm the efficacy of Rovral AquaFlo against seed-borne *Alternaria* spp. and *Botrytis cinerea*.

(A SOLA for the use of the fungicide Apron XL (containing metalaxyl-M) as a seed treatment for ornamental plants was recently secured by HDC (2539/2010). This treatment has potential to reduce seed-borne *Pythium* diseases).

Disinfectant seed treatment

Sodium hypochlorite was used routinely throughout the project for surface disinfection of seed, both in tests to check for the incidence of target pathogens, and also as a comparison treatment in experiments on microwave, steam air and fungicide treatments. Treatment with sodium hypochlorite (1% available chlorine; 5 min soak duration) was in general more effective than the other treatments tested in reducing microbial contamination on seeds and without deleterious effects on seed germination.

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Sodium hypochlorite is not currently approved as a seed treatment (except as a statutory requirement for the treatment of tomato seed). It is recommended that the industry consult with HDC and the Chemicals Regulation Directorate (CRD) as to whether application for a SOLA for use of sodium hypochlorite as a seed treatment, could be warranted.

Aerated-steam treatment

A preliminary experiment using *Tagetes* seed showed that steam air treatments greater than 55ºC reduced germination rates at all treatment durations (10, 20 and 30 minutes), with severe reductions and even total kill at 60ºC and above. Lower temperatures appeared to have little effect on germination irrespective of duration (up to 30 min). However, susceptibility to treatments varied with seed type and different runs of the experiment (due possibly to variation of 1-2ºC from the set temperature). Treatment of commercial seed lots with this equipment would require extensive pre-testing to optimise treatment conditions for individual seed batches. The degree of microbial surface contaminants was not reduced in the steam air treated lupin seed compared to the untreated (control) seed. Some reduction in contamination was observed in the *Tagetes* and *Primula* seed following treatment at 55ºC (10 min). Surface disinfection with 1% bleach solution resulted in the biggest reduction of contamination in both the *Primula* and lupin seed.

The use of precisely conditioned hot humid air for seed treatment has been developed more extensively by Thermoseed in Sweden (www.seedgard.com), whereby optimum conditions for treatment are determined for individual seed batches.

Microwave treatment

Microwave treatment up to 60 sec duration had no adverse effect on germination of *Lobelia*, lupin, *Primula* or *Tagetes* seed. At 120 sec duration and above, germination of lupin was greatly reduced; at 240 sec and above germination of *Primula* was greatly reduced. Germination of *Lobelia* and *Tagetes* seed was unaffected by 240 sec treatment, the longest duration tested. Levels of *Alternaria* sp. on *Tagetes* (8-14% infection) were not reduced by microwave treatment or a sodium hypochlorite treatment. Levels of *Alternaria* sp. on *Lobelia* appeared to be reduced by both microwave and hypochlorite treatment but results were inconsistent. The proportion of ‘clean’ seed of lupin and *Primula* was significantly increased by microwave treatment, but only at durations that also reduced germination. Hypochlorite treatment increased the proportion of ‘clean’ seed of lupin, *Primula* and *Tagetes* species without adversely affecting germination. The adverse effect of microwave treatment on seed germination did not appear related to either seed size or dry weight.

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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 in 2006 was 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

- Be aware of the potential seed-borne origin of key diseases affecting pot plants, bedding plants and cut flowers (Table 1).
- Source seed stocks from reputable sources and ensure the potential known risks from seed-borne pathogens are considered. If necessary get a reassurance from the supplier that they have taken all necessary steps to minimise such risks. In addition, consider an alternative supplier or put in place a suitable fungicide programme to mitigate any risk post-emergence.
- Discuss the availability and use of seed treatments for control of seed-borne pathogens with your seed supplier.
- Consider Rovral AquaFlo dip treatment of seeds for control of seed-borne *Alternaria* spp. and *Botrytis cinerea*. (There is now also a SOLA (2539/2010) available for the use of Apron XL for seed treatment of ornamental crops for the control of *Pythium*).
- Examine plants for disease at an early growth stage, especially those species quite commonly affected by diseases of possible seed-borne origin (e.g. lupin anthracnose, stock *Fusarium* wilt, leaf spot caused by *Alternaria alternata* on *Lobelia*).
- Take action promptly to control any disease of possible seed-borne origin found at an early growth stage; continue monitoring the crop for the disease.

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SCIENCE SECTION

Introduction

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

In year 1 of this project, a comprehensive list was produced of the bedding and pot plant diseases that can originate from use of infected seeds. 29 commercial seed lots of 18 ornamental species were tested and the suspected 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, literature on chemical and non-chemical methods for control of seed-borne pathogens was reviewed, and promising treatments were selected for testing in year 3. 25 commercial seed lots across 15 ornamental species were also tested in year 2 the suspected 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*, germanium and salvia. Isolates of *Alternaria* from *Alyssum* and *Zinnia* and bacteria from several hosts did not cause damage in pathogenicity tests.

In year 3 of the project, the objectives were to:

- Determine the levels of seed-borne fungi and bacteria in up to 30 commercial seed lots;
- Test the pathogenicity of selected fungi and bacteria isolated from seeds by inoculation of young plants;
- Test some potential chemical and non-chemical treatments for their effect on some seed-borne pathogens and seed germination;
- Produce the text and images for a factsheet on seed-borne diseases of ornamentals.

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1. Prevalence of seed-borne fungi and bacteria

Introduction

In project years 1 and 2, seed was sourced via plant propagators largely from two seed-houses and was, on the whole, clean of target pathogens. However, growers tend to use a range of seed companies and to reflect this, it was decided to source seed from a more diverse selection of seed companies, who also provide seed to commercial nurseries. STC therefore obtained seeds from four commercial suppliers for tests in Year 3. At ADAS, all seeds were obtained from a single seed-house (as in previous years), except for lupin seed which was sourced from three additional suppliers.

Materials and methods

Methods used for detection of seed-borne fungal and bacterial pathogens

1. Sample size

Three hundred seeds per lot were tested, both with and without surface sterilisation. If the true level of a seed-borne infection is 1%, a random sample of 299 seeds is required to give a 95% probability of detecting culturable fungi and bacteria, providing an appropriate method and growth medium is used, and providing the organism of interest is not swamped by other micro-organisms on the seed.

It is recognised that levels of seed infection less than 1% can give rise to serious disease problems. For example, infection of lupin seed with *Colletotrichum gloeosporioides* at 0.1% can cause yield losses of up to 50%. Similarly, *Xanthomonas campestris* pv. *campestris* on brassica seed at less than 1% can cause serious crop losses if conditions are conducive to spread during plant propagation. However, for the purposes of this project, a sample of 300 seeds was used as a reasonable practical number to examine when large numbers of seed lots are to be tested.

2. Visual examination of dry seed

- The occurrence of any pelleting or chemical seed treatment present on seeds, was noted.

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- Seeds were spread thinly in a white tray and examined for whole or fragments of *Sclerotinia sclerotiorum.* Samples of any suspect sclerotia of *S. sclerotiorum* or other sclerotial fungus were plated onto PDA + streptomycin for identification.

- Seeds were examined under a binocular microscope and the occurrence of any pycnidia (e.g. *Septoria*), acervuli (e.g. *Colletotrichum*), sclerotia on the seed surface or submerged in the seed coat; or individual spores (e.g. rust) or spore masses; or blemishes on the seed (e.g. *Pseudomonas*) were noted. Where a fruiting structure or spore was observed, a slide was prepared and examined under the microscope (Mathur *et al*., 2003).

3. Examination of seed by agar plate method

Growth media

Wherever possible, a growth medium selective for the main pathogen of interest on a particular ornamental species was used. This reduced the likelihood of saprophytic micro-organisms on the seed (e.g. *Penicillium, Mucor* and *Rhizopus*), from preventing outgrowth of the organism of interest. Validated methods published by the International Seed Testing Association were used where these were available for fungal or bacterial genera of interest to this project. The media used are listed in Table 1.1.

Table 1.1: Agar media used for plating of ornamental seed species

Pathogen	**Medium**	**Reference**
Alternaria species	Malt agar (25%)	Anon., 2003a,b; 2005a
Botrytis cinerea	Malt agar or Botrytis Selective Medium (BSM)	Anon., 2002b; 2005b
Colletotrichum sp.	Malt agar	Anon 2005b
Fusarium oxysporum	Komada's medium	Singleton *et al.,* 1992
Ramularia species	Malt agar	
Septoria species	Malt agar + 100 ppm strep	Anon., 2002a
Pseudomonas	Potato Dextrose Agar	Neergard, 1979
Xanthomonas	1% glucose or dextrose agar	Neergard 1979

In order to exclude the possibility of cross-contamination between seed samples, all equipment, surfaces etc were disinfected between samples by spraying 70% ethanol. Seed were placed on agar media using standard aseptic procedures.

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Surface sterilisation

Sodium hypochlorite was used for surface sterilisation of seed, diluted to 1% available chlorine. The following formula was used to take account of the variation in chlorine concentration in commercial bleach or stock solution:

$$V_{stock} = V_{final} \times C_{final} / C_{stock}$$

e.g. to prepare 100 ml solution of 1% available chlorine from a stock containing 12% available chlorine:

$V_{stock} = 100 \times {}^{1}/_{12} = 8.3$
Thus, add 8.3 ml stock to 91.7 ml of water.

One 300 seed sample from each 600 seed lot was pre-treated by soaking in 1% available chlorine for 5 mins; seeds were plated out immediately, after drying in a sterile airflow, without rinsing in water (Neergard, 1979).

Incubation

Plated seeds were incubated for 5-7 days at 18-25º, and fungi growing out from seeds onto the agar were examined and identified. Lupin seeds were incubated for at least 21 days.

4. Pathogenicity testing

A standard protocol was prepared for use by ADAS and STC personnel for pathogenicity testing and this is included in Appendix.

5. Pathogen identification

Where fungal or bacterial colonies were isolated that were suspected as plant pathogens, they were first purified by repeated sub-culturing to ensure freedom from any secondary organisms.

For seed batches tested where bacterial species were the target pathogen, representative cultures of bacteria isolated were sent to CSL for identification by Fatty Acid Profile analysis and by observation of typical growth characteristics on relevant growth media.

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Problems with fast-growing saprophytes (mainly *Mucor*) over-growing plates was overcome using agar amended with Dichloran-B to inhibit growth of *Mucor* species, while allowing growth of other species. A total of 32 seed lots was tested, 17 by ADAS and 15 by STC.

Results and discussion

Results of the samples tested by ADAS are shown in Table 1.2. No seed-dressing was visible on any of the seeds except for lupin (5). A range of saprophytic fungi including *Cladosporium* sp., *Penicillium* sp. and particularly *Mucor* and *Rhizopus* spp., were recovered from the majority of seed batches tested which accounts for the differences between data for % seeds with suspected pathogenic contamination and % clean seeds. Contamination was particularly high on *Lychnis* and *Primula* batch 2, with over 98% seeds infested in both batches. The exceptions were *Aquilegia*, *Impatiens*, *Lobelia* and lupin batch 4&5, where over 80% 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, using chemical disinfection or physical treatment. After treatment with sodium hypochlorite, over 70% of seeds were 'clean' in most batches. An exception was *Primula* batch 2, for which the incidence of contamination was 44% even on surface disinfected seed, due to a combination of bacteria, *Cladosporium*, *Penicillium*, *Fusarium* sp. and other fungi.

Suspected plant pathogens were recovered from the following: *Aquilegia* (*Pythium* sp. and Alternaria sp.), *Cyclamen* (*Fusarium oxysporum*), *Geranium* (bacterial species), *Lobelia* (*Alternaria* sp.), *Lychnis* (*Phoma* sp.), *Primula* (*Botrytis cinerea*), stock (*Fusarium* sp./ *Pythium* sp.), and *Zinnia* (*Alternaria* sp./ *Botrytis cinerea*). Five batches of lupin seeds were tested (sourced from four different seed-houses) but *Colletotrichum acutatum* was not recovered from any batch (at the levels tested). Representative isolates of *Alternaria* species from *Zinnia* were examined microscopically but did not conform to the published description for *A. zinniae* (Ellis& Ellis, 1985); isolates were, however, maintained for pathogenicity tests. The highest incidences of target pathogens were isolated from *Lychnis* (*Phoma* sp.) and *Pelargonium* (bacterial species). In both cases, outbreaks of *Phoma* sp. on *Lychnis* and bacterial leaf spot on *Pelargonium* were reported from the source nursery in 2009 (not necessarily from the same seed batches). Typical isolates of bacterial species from *Pelargonium* seed were sent to FERA for fatty acid profiling; a range of *Bacillus* and *Brevibacillus* species (none known as pathogenic to *Geranium*/*Pelargonium*) were identified. *Xanthomonas* and *Pseudomonas* species were not identified, although these may have been outgrown on original isolation plates. A second batch of *Pelargonium* seed was sent to

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FERA to test for bacterial species by isolation onto a range of semi-selective and non-selective media and no *Xanthomonas* spp., *Pseudomonas* spp. or other bacterial pathogens were isolated (results not included in Table 1.2).

Results of the seed testing at STC are shown in Table 1.3. A range of saprophytic fungi were recovered, similar to the results from ADAS. Contamination was high on *Antirrhinum*, *Lobelia*, *Rudbeckia* and *Verbena* with ≥98% of seeds affected. Conversely, *Cineraria* and *Zinnia* had over 80% of clean seeds. After treatment with sodium hypochlorite, over 90% of seeds were 'clean' in most batches, the exceptions being *Aquilegia*, lupin, *Pelargonium, Rudbeckia*, *Verbena,* wallflower and *Zinnia*.

In terms of suspected plant pathogens, *Alternaria* spp. were recovered from *Aquilegia*, Cineraria, *Lobelia*, *Rudbeckia*, *Verbena* and *Zinnia*, and *Botrytis cinerea* was recovered from *Primula*. The highest incidence of *Alternaria* sp. was 28% on *Verbena*; the highest incidence of *B. cinerea* was 3% on *Primula*. Isolates of target pathogens from various hosts were maintained for pathogenicity tests (see section 2).

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Table 1.2: Recovery of fungi and bacteria from ornamental seeds – ADAS tests, Year 3

Crop	Target plant pathogen(s)	% of seeds from which suspected plant pathogens were recovered (300 seed tested)		% of 'clean' seeds	
		Surface disinfected	Not surface disinfected	Surface disinfected	Not surface disinfected
1. *Aquilegia*	*Alternaria* sp. / Pythium *sp.*	0.0	2.3	99.7	96.3
2. *Cyclamen*	*Fusarium oxysporum*	0.0	5.3	78.2	68.8
3. *Impatiens*	*Pythium* sp.	0.0	0.0	100.0	81.3
4. *Lobelia*	*Alternaria* sp.	0.3	1.0	94.7	97.7
5. Lupin 1.	*Colletotrichum acutatum*	0.0	0.0	89.3	23.1
6. Lupin 2.	*Colletotrichum acutatum*	0.0	0.0	75.7	68.0
7. Lupin 3.	*Colletotrichum acutatum*	0.0	0.0	89.3	50.0
8. Lupin 4.	*Colletotrichum acutatum*	0.0	0.0	93.3	82.3
9. Lupin 5.	*Colletotrichum acutatum*	0.0	0.0	96.0	92.6
10. *Lychnis*	*Phoma sp.*	9.7	21.3	69.0	0.0
11. Pansy	*Ramularia lactea* / *R. agrestis*	0.0	0.0	84.7	26.3
12. *Pelargonium**	*Pseudomonas* sp. / *Xanthomonas* sp.	(3.3)	(43.0)	93.0	25.3
13. *Phlox*	*Septoria drummondii*	0.0	0.0	72.0	14.0
14. *Primula* 1.	*Botrytis cinerea*	0.0	2.0	91.7	40.0
15. *Primula* 2.	*Botrytis cinerea*	0.7	0.3	55.7	1.7
16. Stock	*Fusarium* sp.	0.3	1.0	73.0	26.3
	Pythium sp.	0.3	0.7	-	-
17. *Zinnia*	*Alternaria* sp. / *Botrytis cinerea*	3.3	1.7	81.7	50.7

Notes:
Aquilegia: - *Alternaria* found, no *Pythium*
Stock: - *Fusarium* & *Pythium* found on surface sterilised and non-surface sterilised seed
Zinnia: - *Alternaria* on surface sterilised and non-surface sterilised seed, *Botrytis* on surface sterilised seed only
'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.
*150 seed tested; ( ) - bacterial colonies; no plant pathogenic bacteria were identified

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Table 1.3: Recovery of fungi and bacteria from ornamental seeds – STC tests, Year 3

Crop and supplier (coded A-D)	Target plant pathogen(s)	% seeds from which suspect plant pathogens recovered (300 seed tested)		% of 'clean' seeds (of 300)	
		Surface disinfected	Not surface disinfected	Surface disinfected	Not surface disinfected
1. *Antirrhinum* (C)	*Pseudomonas syringae*	0	0	93	1
2. *Aquilegia* (D)	*Alternaria* and *Pythium*	0	0	-	-
3. *Cineraria* (B)	Alternaria cinerariae	0	0	98	88
4. *Lobelia* (A)	*Alternaria alternata* and *Sclerotinia sclerotiorum*	0 Alternaria, 0 Sclerotinia	3 Alternaria, 0 Sclerotinia	91	0
5. Lupin (C)	*Colletotrichum acutatum*	0	0	78	8
6. Pansy-1 (D)	*Ramularia* spp.	0	0	89	4
7. Pansy-2 (A)	*Ramularia lactea* and *R. agrestis*	0	0	92	5
8. *Pelargonium* (B)	*Pseudomonas* and *Xanthomonas* spp.	0	0	78	18
9. *Petunia* (D)	*Sclerotinia*	0	0	98	40
10. *Phlox* (B)	*Septoria drummondii*	0	0	90	30
11. *Primula* (C)	*Botrytis cinerea*	0	2.6	96	14
12. *Rudbeckia* (D)	*Alternaria* spp.	1.2	0.8	59	2
13. *Verbena* (D)	*Alternaria* and *Phoma* sp.	17 0	28.4 0	61 -	0 -
14. Wallflower (B)	*Xanthomonas campestris*	0	0	51	4
15. *Zinnia* (A)	*Alternaria* and *Botrytis cinerea*	5 *Alternaria,* 0 *Botrytis*	5 *Alternaria*, 0 *Botrytis*	79	84

* Bacterial isolates are testing for their pathogenicity to the seedlings
^ Isolates subbed to check and confirm

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2. Pathogenicity tests with fungi recovered from seed

2.1 Introduction

Isolates of selected fungi and bacteria recovered from seeds were tested for their ability to cause disease in plants of the species from which they were obtained. The isolates used were ones identified as suspect fungal or bacterial pathogens; where available, they were taken from surface disinfected seed in order to minimise the chance of selecting saprophytic isolates. Isolates were sub-cultured to check for purity and then grown on agar for production of bacterial cells, fungal spores or fungal mycelium for use in pathogenicity tests.

2.2 ADAS tests

A summary of the fungi recovered from seeds by ADAS and used in pathogenicity tests is shown in Table 2.1.

Table 2.1: Fungi recovered and used for pathogenicity testing in Year 3 of the project (ADAS)

Species	Target pathogen	Number of isolates tested	% occurrence on seeds
*Cyclamen**	*Fusarium oxysporum*	2	14
Cyclamen	*Fusarium oxysporum*	1	5
Lobelia	*Alternaria* sp.	1	4
Lychnis	*Phoma* sp.	1	31
Primula	*Botrytis cinerea*	6	3
*Tagetes**	*Alternaria sp.*	3	60
*Zinnia**	*Alternaria* sp., *Botrytis* sp.	2 *Alternaria* sp., 2 *Botrytis* sp.	38
Zinnia	*Alternaria* sp., *Botrytis* sp.	2 *Alternaria* sp., 2 *Botrytis* sp.	5

* recovered from seed tested in Year 2.

The fungi were isolated into a pure culture by removing sections of the leading edge of the target pathogen colonies on seed isolation plates onto PDA + S or oatmeal agar (*Phoma* sp.). Fungi were grown at 20ºC in an incubator with a 12 hour light and 12 hour dark cycle.

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2.2.1 Cyclamen

Root drench inoculation

A *F. oxysporum* spore suspension of 7 x 10^6 spores / ml was prepared and 100 ml was poured onto the base and corms of ten potted young *Cyclamen* plants, giving 7 x 10^5 spores per plant. These were then placed on capillary matting and allowed to grow up in a glasshouse. The capillary matting was watered in order to avoid water splash. Control plants were drenched with sterile distilled water (SDW).

Plants were destructively assessed 60 days after inoculation. No staining was found in either the control or inoculated corms, and no symptoms were seen on the plants or roots.

Root dip inoculation

A spore suspension of above 1 x 10^4 spores/ml was prepared for each of two isolates of *F. oxysporum* recovered from *Cyclamen* seed. The base of 20 young plug plants of *Cyclamen* cv. Halios Flame were placed in the spore suspension in a tray for 1 hour. As a control, plants were placed in SDW for an hour. The plants were then potted on and allowed to grow in a greenhouse with overhead watering for 3 months. Any diseased plants were removed and examined as disease expression became apparent.

At 7 days after inoculation, one wilting plant occurred in each of the treatments inoculated with *F. oxysporum*. The corms were found to be grey but with no staining of the vascular tissue. The diseased tissue was plated onto PDA + S and *Fusarium* sp. spores were recovered. After 18 days, four wilting plants occurred in the control treatment. No *Fusarium* sp. was recovered from corms and it is thought that wilting was due to *Pythium* root rot.

After three months, remaining plants were destructively assessed. Although all of the plants had a healthy appearance, in some corms there was vascular staining suggestive of fusarium wilt (Table 2.2). The two treatments which were inoculated with *F. oxysporum* had a higher incidence of vascular staining, and *F. oxysporum* was only recovered from inoculated plants. The results of this test indicate that the two isolates of *F. oxysporum* recovered from *Cyclamen* seed are pathogenic to *Cyclamen* plants.

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Table 2.2: Effect of *Cyclamen* root dip inoculation with two isolates of *F. oxysporum* recovered from *Cyclamen* seed on occurrence of corm symptoms on remaining plants after 12 weeks

Treatment	Stained vascular bund in corm		Recovery of *Fusarium* from corms
	No. of affected plants / total no. assessed	%	
1. SDW (control)	3/16	19	0/16
2. *F. oxysporum* (isolate 1)	8/19	42	2/19
3. *F. oxysporum* (isolate 2)	5/19	26	2/19

2.2.2 Lobelia

An isolate of *Alternaria* sp. obtained from *Lobelia* seed was tested by spray inoculation with a spore suspension (1 x 10^6 spores/ml) onto recently germinated seedlings

Eight germination boxes were cleaned using 90% ethanol in order to eliminate any fungal contamination. A piece of filter paper was then placed in the bottom of each and 50 ml of SDW was added. Excess water was decanted and a pinch of *Lobelia* seeds was spread onto the paper and allowed to germinate. Four boxes of seedlings were each inoculated with the isolate of *Alternaria*; four boxes were inoculated with SDW as a control. The sealed boxes were incubated at ambient temperature in the laboratory.

At 7 days after inoculation, the proportion of collapsed *Lobelia* seedlings and ungerminated seeds was significantly higher (P<0.001) for inoculated compared with uninoculated seedlings (using generalised linear models in Genstat) (Table 2.3). This finding demonstrated that the *Alternaria* sp. found on *Lobelia* seed was pathogenic to *Lobelia* plants.

Table 2.3: Effect of inoculation with *Alternaria* sp. (*ex Lobelia* seed) on *Lobelia* seedling health

Treatment	% germinated healthy seedlings	% ungerminated seeds	% collapsed seedlings
Uninoculated control	77.2 (2.7)	22.4 (2.2)	0.4 (0.3)
Inoculated	38.8 (3.1)	45.4 (2.5)	15.8 (1.8)
D.f.	38	38	38
F. probability	<0.001	<0.001	<0.001

() standard error

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2.2.3 Lychnis

An isolate of *Phoma* sp. obtained from *Lychnis* seed was grown on oatmeal agar. Although fungal bodies consistent with pycnidia developed in culture, no spores were obtained from them despite prolonged incubation. Inoculation was therefore carried out using mycelial plugs (5 mm diameter) on agar. Mycelium was placed downwards onto intact or wounded leaves of potted *Lychnis* plants. Leaves were wounded using a sterile needle. Intact and wounded leaves were inoculated with oatmeal agar as a control. Each treatment was applied to 5 plants with 4 inoculation sites per plant. Trays of inoculated plants were placed in clear polythene bags and misted with SDW before the bags were loosely closed. After 6 days, bags were opened and misted daily to keep humid conditions. After 14 days the plants were assessed for lesion diameter.

The greatest incidence of spreading lesions developed on wounded leaves inoculated with *Phoma* sp. (Table 2.4). Occasional lesions developed on unwounded leaves and on the control plants. These results indicate that the *Phoma* sp. recovered from *Lychnis* seed is a weak pathogen of *Lychnis* leaves under the conditions of this pathogenicity test.

Table 2.4: Effect of inoculation of *Lychnis* leaves with two isolates of *Phoma* sp. recovered from *Lychnis* seed on occurrence of lesions after 14 days

Treatment	Number of inoculation sites (of 20) with:		Mean lesion diameter (mm)
	Limited lesions (<5mm)	Spreading lesions (>5 mm)	
1. Unwounded control	1	0	2.0
2. Wounded control	0	2	16.5
3. Unwounded inoculated	0	1	10.0
4. Wounded inoculated	2	8	10.3

2.2.4 Primula

Six *B. cinerea* isolates recovered from two batches of *Primula* seed were tested. Seven fully grown plants obtained from a commercial nursery were inoculated with mycelial 8 mm diameter plugs on PDA+S using one plant for each isolate and a control (agar alone). There

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were six inoculation sites (6 leaves) per plant, three of them wounded and three unwounded. Plants were placed in polythene bags for 48 hours and leaves were assessed after 7, 14, and 28 days.

All six isolates caused symptoms to develop, including spreading lesions on leaves, necrosis of the petals and mycelial growth at the stem base (Table 2.5). Grey mould symptoms also developed on the uninoculated controls. This may have been due to spread from inoculated plants, development of latent *Botrytis* from within the plant, or stimulation of *Botrytis* spores on plants by addition of the nutrient agar plugs.

Table 2.5: Pathogenicity of six isolates of *B. cinerea* obtained from *Primula* seed to *Primula* leaves

Treatment (isolate)	**No. of inoculation sites (of 3) with spreading lesions after 7 days**		**Mean lesion diameter (mm) after 28 days**	
	Unwounded	**Wounded**	**Unwounded**	**Wounded**
1. Control – agar	2	1	45	57
2. *B. cinerea* (1)	1	2	39	16
3. *B. cinerea* (2)	3	3	46	62
4. *B. cinerea* (3)	2	2	42	57
5. *B. cinerea* (4)	3	3	84	78
6. *B. cinerea* (6)	3	3	78	97
7. *B. cinerea* (20)	0	2	0	72

All plants showed symptoms of grey mould to varying extents. A greater proportion of wounded leaves than unwounded leaves died back following inoculation.

The inoculated control plants also showed lesions on 66.6% of the leaves. This could be due to spread from inoculated plants. Another explanation could be that the *Botrytis* sp. was latent within the plant and the extra nutrition from the agar may have caused the fungus to become active and cause disease within the plant. In summary, tests to determine the pathogenicity of *B. cinerea* isolates obtained from *Primula* seed to *Primula* plants was inconclusive.

2.2.5 Tagetes

Sixteen young *Tagetes* plants were potted into new 9 cm pots containing Levington M3 compost. These were then grown in a polytunnel with overhead watering for 3 weeks.

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Spore suspensions were prepared from 27 day old plates of *Alternaria* sp. isolated from *Tagetes* seeds on Malt Extract Agar at 20-22ºC. Spores were collected by drenching the plates in SDW, scraping, and filtering through muslin. There were three different isolates tested. The concentrations achieved, determined using a haemocytometer, were 4 x 10^4, 2 x 10^5 and 1 x 10^4 spores per ml respectively. Four plants were placed into plastic bags and inoculated with approximately 20 ml of spore solution (until run off) using a hand mister for each isolate, as well as another 4 which were sprayed with SDW to act as a control. All plastic bags were sealed, in order to achieve a high humidity, and placed into a controlled environmental cabinet (set at 20ºC, 12 h light / 12 h dark) for eleven days before assessment for leaf spotting.

Few leaf spots suggestive of *Alternaria* sp. infection developed, although there were necrotic areas on plants. Many young flower buds became dark brown or black in appearance, especially on inoculated plants. Growth of *Alternaria* sp. was visible on these buds and a count was done to determine the proportion affected (Table 2.6) which was much greater on inoculated plants (75-100%) than on the uninoculated control (17%). In summary, this test showed that isolates of *Alternaria* sp. obtained from *Tagetes* seed were pathogenic to *Tagetes.*

Table 2.6: Occurrence of black buds on *Tagetes* infected with *Alternaria* sp. following spray inoculation with three different isolates of the fungus obtained from *Tagetes* seed

Treatment (isolate number)	**Mean number of black buds per plant**	**Mean number of black buds infected with *Alternaria* sp.**	**Mean percentage of black buds infected (%)**
Control	1.5	0.3	17
Alternaria sp. (1)	2.0	1.5	75
Alternaria sp. (2)	0.8	0.8	100
Alternaria sp. (3)	2.5	2.3	90

2.2.6 Zinnia

Two isolates each of *B. cinerea* and *Alternaria* sp. were recovered from *Zinnia* seed during the second year of the project. Two young *Zinnia* plants per isolate were spray-inoculated, each with spore suspension made from 27 day old cultures on malt extract agar (MEA). The concentration used was 2 x 10^5 spores per ml. One plant was placed in a plastic bag and inoculated with approximately 20 ml of spore solution (until run off) using a hand mister.

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Another plant was sprayed with SDW and placed in a plastic bag to act as a control. These were then placed in a controlled environmental cabinet for eleven days.

In the third year of the project two isolates of *Botrytis cinerea* and two of *Alternaria* sp. were recovered from *Zinnia* seed. Mycelial plugs were used to compare the pathogenicity of these using PDA+S agar plugs as a control. Five plants were inoculated, one for each isolate and the control. Six leaves per plant were inoculated, three directly and three after wounding with a sterile mounted needle. Plants were placed in polythene bags and misted with distilled water in order to create an environment with high relative humidity (RH). After 48 hours the bags were opened at the top.

Using the same pure cultures a spray-inoculation was performed. The concentrations for each isolate ranged from 5×10^5 to 1×10^6 spores/ml. Two *Zinnia* plants were sprayed until run off with each isolate before being put into a polythene bag which had been sprayed with SDW. This was compared with two plants which had been sprayed with SDW until run off. After 48 hours the bags were opened. Plants were examined for symptoms and lesions measured at 14 days after inoculation.

All isolates were pathogenic to *Zinnia* from mycelial inocula (Table 2.7). The two isolates of *Alternaria* sp. were only able to infect wounded leaves. The spray inoculation test also indicated that the isolates were pathogenic. Nearly all plants inoculated with *Alternaria* sp. or *B. cinerea* showed a number of lesions while the controls showed no disease symptoms. These results indicate that both *Alternaria* sp. and *Botrytis cinerea* obtained from *Zinnia* seed were pathogenic to *Zinnia* leaves.

Table 2.7: Effect of inoculation of *Zinnia* with *Alternaria* sp. and *Botrytis cinerea* recovered from *Zinnia* seed

Treatment	Mean lesion diameter (mm) after mycelial inoculation of:		Number of plants (of 2) with leaf lesions after spray inoculation
	Unwounded leaves	Wounded leaves	
1. Untreated	0	0	0
2. *Alternaria* sp.	0	1	2
3. *Alternaria* sp.	0	1.3	1
4. *B. cinerea*	0	1.6	2
5. *B. cinerea*	8	4.3	1

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2.3 STC tests

The methods used for pathogenicity tests at STC followed those used in Year 2 (see Appendix 1).

2.3.1 Bacterial isolates on Aquilegia

A total of four bacterial isolates were tested on *Aquilegia* seedlings. A turbid suspension of each of the unidentified bacteria was applied as droplets to the foliage of seedlings both with and without damage. The inoculated seedlings were then incubated in seedling germinators alongside untreated damaged and undamaged control seedlings. None of the isolates tested resulted in any symptoms or lesions developing, indicating the isolates were likely not pathogenic to *Aquilegia*.

2.3.2 Alternaria on Verbena

Three morphologically different isolates of *Alternaria* sp. were tested on young *Verbena* seedlings. A spore suspension was sprayed onto damaged and undamaged seedlings prior to incubation. No symptoms consistent with an *Alternaria* infection were observed on the seedlings and the isolates were deemed non-pathogenic.

2.3.3 Bacterial isolates on Antirrhinum, Pelargonium and wallflower

Unidentified bacterial isolates collected from *Antirrhinum*, *Pelargonium* and wallflower were tested on seedlings of the same host. Droplets of each of the suspended bacterial isolates were placed on the leaves of the undamaged plants, other plants were stab inoculated into the stem with the bacterial isolate. All inoculated plants were incubated in plastic bags alongside uninoculated control plants. Tests on *Pelargonium* and wallflower were negative. One isolate of a bacterium produced symptoms on the damaged *Antirrhinum* seedlings. However, FAP tests did not identify any plant pathogenic bacteria, so it is unlikely that symptoms were due to the bacterium. The best matches on two cultures tested were *Ochrobacter anthropi* (similarity index 0.579) and *Alcaligenes faecalis* (similarity index 0.817) (FERA reference 20914461-2). It was concluded that the bacteria isolated from *Antirrhinum*, *Pelargonium* and wallflower were not pathogenic to these hosts.

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2.3.4 Alternaria, Ulocladium and bacteria on Cineraria

Two *Alternaria* sp. isolates collected from *Cineraria* seed were tested on young seedlings. The isolates were inoculated onto seedling leaves on small agar pieces with and without damage to the leaves. Although both isolates resulted in the formation of lesions on both the undamaged and damaged leaves, close examination of the spore type showed neither isolate conformed to *A. cinerariae* or *A. denisii*. The results of this test were therefore inconclusive.

The pathogenicity of two isolates of bacteria and an *Ulocladium* sp. was also tested. None were found to be pathogenic.

2.3.5 Alternaria and Fusarium on Lobelia

An isolate of *Alternaria* sp. and two *Fusarium* sp. isolates were tested on young seedlings. The *Alternaria* sp. produced lesions on both the damaged and undamaged seedlings and was considered pathogenic. Colony and spore morphology was considered consistent with *A. tenuissima.* One of the *Fusarium* sp. isolates resulted in severe lesions on both the damaged and undamaged leaves resulting in complete plant collapse.

2.3.6 Alternaria on lupin

An isolate of *Alternaria* sp. which was detected on 6% of the non-surface sterilised seed was tested on young seedlings and found to be pathogenic on both damaged and undamaged seedlings.

2.3.7 Alternaria on phlox

The pathogenicity of several isolates of *Alternaria* sp. was tested on young phlox seedlings. All were found to be pathogenic to some degree, resulting in the formation of lesions on the inoculated leaves.

2.3.8 Botrytis on Primula

An isolate of *B. cinerea* found on 2.6% of the non-surface sterilised *Primula* seed was found to be pathogenic on young seedlings.

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2.3.9 Alternaria on Zinnia

Five isolates of *Alternaria* sp. collected from both surface sterilised and non-sterilised seed were tested. All were found to be pathogenic to some degree. However, the morphology of the colonies and spores was not consistent with *A. zinniae*.

3. Sourcing infected seed batches

Introduction

Because of difficulties in sourcing seed lots that were naturally infested with target pathogens, studies were carried out to determine the potential for generating infested seed lots using artificial inoculation.

Materials and methods

Lupin

Five flowering lupin plants were inoculated by spraying to run-off with a spore suspension of *Colletotrichum acutatum* (*ex* lupin). The flowerheads were sealed in a polythene bag for approximately 48 h, in order to maintain high relative humidity. Subsequently, the plants were maintained in a polytunnel with overhead watering. After 3 weeks, seed pods had developed on the plants and were removed.

Seed collected from the pods (36 in total) was plated onto PDA+S, half with and half without surface sterilisation (1% sodium hypochlorite solution). Plates were incubated at 20^{o}C under UV light for 2 weeks.

Primula

Primula were inoculated using dry spores and a spore suspension in SDW. For each method, 20 flowering plants were used. A clean dry paint brush was used to dry-dust spores from sporulating cultures of *B. cinerea* (*ex Primula*) onto the centre of each flower. The plants were placed for 24 h inside clear polythene bags that had been sprayed inside with a fine mist of distilled water. Plants were subsequently monitored for *Botrytis* development and seed formation. For spray-inoculation, sporulating cultures of *B. cinerea* were prepared using a spore suspension (1 x 10^5 spores/ml) containing 0.1% Tween 80 in SDW. The

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flowers of 20 *Primula* plants were sprayed with the spore suspension to the point of run-off, using a hand-held mister. The plants were placed within sealed bags for 4 h, then maintained and monitored for *Botrytis* development and seed formation. Once seed had developed, mature seed cases were collected from the plants and stored in labelled paper bags in the laboratory.

Seed from both inoculation techniques was checked for the internal and external incidence of *B. cinerea*. Half of the seeds were surface disinfected (5 min in 1% sodium hypochlorite), dried in a laminar flow cabinet, and then plated onto PDA+S using aseptic procedures. The other half of the seeds were plated onto PDA+S without treatment. The incidence of *B. cinerea* was determined after 7 and 10 days inoculation at 20^{o}C.

Results and discussion

Lupin

Mycelium and spores of *C. acutatum* was confirmed on the surface of seed pods taken from inoculated lupin flowers (Figure 3.1). Fifteen out of 18 non-surface disinfected seeds and five out of 18 surface disinfected seeds tested positive for *C. acutatum.* These results indicated that artificial inoculation of lupin flowers could be used to produce seed batches with mainly surface-borne infection by *C. acutatum*, but many plants would be required to provide sufficient seed numbers for replicated seed treatment experiments.

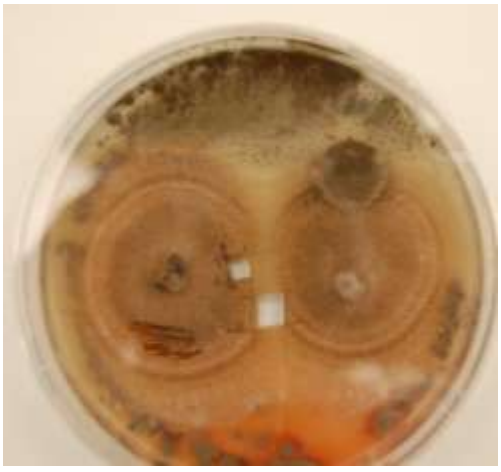

Figure 3.1: a) Lupin pods affected by *C. Acutatum* b) Growth of *C. acutatum* from lupin seed

Primula

The fungus was recovered after both spray inoculation and dry dusting (Table 3.1). Infection was mainly surface-borne. As with lupin, many plants would be required to produce

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sufficient infected seed for seed treatment experiments. Artificial inoculation using the methods described is therefore unlikely to be a viable option for production of large batches of infected seed.

Table 3.1: A comparison of spray inoculation and dry-spore inoculation of *Primula* flowers with *B. cinerea* on occurrence of seed-borne infection by the fungus

Inoculation method	**% seed infected with *B. cinerea***	
	Non disinfected seed	**Surface disinfected seed**
Spore suspension in water, spray inoculated	25	0
Spore powder, dry dusted	11	17

4. Effects of microwave treatments on seed-borne fungi

Introduction

The objectives of the study were:

1. To determine the effect of microwave treatments on the germination of seed of three selected ornamental species (lupin, *Tagetes* and *Primula*), representing three different seed sizes (large, medium and small respectively).

2. To determine the effect of microwave treatments on microbial contamination and target pathogen (where present) of seed of the selected ornamental species.

3. Based on results from objectives 1 and 2, modify treatment conditions to determine the effect of microwaving on levels of *Alternaria* spp. and microbial contaminants on *Lobelia* seed (using a seed batch reported to be infested with *Alternaria* spp.).

Materials and methods

Treatments

Seed batches of lupin, *Tagetes* and *Primula* were used that were known to have microbial contamination but not necessarily of the target pathogen. Each seed batch was microwaved for the following durations using an 800 W microwave with turn-table (Panasonic NN5452B):

1. Nil treatment

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2. Microwaved for 30 sec
3. Microwaved for 60 sec
4. Microwaved for 120 sec
5. Microwaved for 240 sec
6. Sodium hypochlorite treatment (5 mins in 1% available chlorine)

Initially, the microwave was run on full power for 5 min containing only a plastic beaker with 400 ml tap water. Subsequently, each replicate seed sample was treated separately, according to the treatment durations listed above. Seeds were treated as a single layer on a labelled paper plate and were visually dry. The microwave oven also contained a beaker of cold tap water (400 ml), replaced for each treatment. This was following a procedure previously reported for microwave treatment of seed. After treatment, the seeds were cooled in a laminar flow cabinet prior to setting up germination or plate tests.

Microwave treatment was compared with no treatment (untreated control) and a standard surface disinfection treatment in sodium hypochlorite (1% available chlorine) for 5 min. After sodium hypochlorite treatment, excess liquid was decanted off and seeds were left to dry in a laminar flow cabinet.

Based on initial results a batch of *Lobelia* seed reported to be infested with *Alternaria* spp. was treated using the following conditions:

1. Nil
2. Microwaved for 60 sec
3. Microwaved for 120 sec
4. Microwaved for 240 sec
5. Sodium hypochlorite treatment (5 mins in 1% available chlorine)

Experimental design and statistical analyses

For each seed batch, three replicate samples of 100 seeds were used. Seed germination and microbial contamination tests were carried out on 50 seeds per replicate. For the *Lobelia* seed, 100 seeds per replicate were used from treatments 1 and 5, 50 seeds per replicate for other treatments.

For each species, seed batches were sub-sampled using the spoon method (Mathur & Kongsdal, 2003) to give 18 sub-samples (6 treatments x 3 replicates) each of 5 g. Each sample was placed in a clean Petri dish labelled with treatment and replicate number.

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The effect of microwave treatments on seed germination and the incidence of microbial contamination were determined by generalised linear models in Genstat. Data for each species were analysed separately.

Seed germination tests

Seed germination boxes were prepared by inserting a pleated filter paper in a plastic box, to give 50 pleats. A sheet of wrapper filter paper was placed around the filter paper pleat so that it was overlapping on top. Tap water (50 ml) was added to each box, which was then left for at least 2 hours before adding any seeds. After treatment of each replicate seed batch, one row of 50 seeds was placed in a box (3 rows per treatment in a single box, 150 seeds in total). The lids were replaced and boxes incubated at 20^{o}C (16 h light / 8 h dark) in an incubator or controlled environment cabinet. Seed germination was assessed after 7 and 14 days using the following categories:

Normal: shoots green, with 2 cotyledons and no visible disease.
Abnormal: abnormalities include:

- Badly diseased
- Discoloured shoot
- Distorted shoot
- Missing a cotyledon

Fresh seed: seeds which remained firm and apparently viable at the end of the test were classified as fresh ungerminated seed and are reported separately from the percentage germination.

Dead seed: seeds which at the end of the test period were either decayed, mouldy or soft or had not produced any seedling or part of a seedling and were not fresh, were classified as dead seeds.

Incidence of microbial contamination

After treatment, 50 seeds were plated onto PDA+S, 10 seeds/plate, using aseptic procedures. Tweezers were dipped in ethanol and flame sterilised every 5 seeds. Tweezer tips were allowed to cool before picking up seed. Plates were incubated at 20^{o}C in the dark. The incidence of fungal and bacterial growth from each seed was assessed 7 and 14 days after plating.

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Dry weights

Dry weights of seeds used in these experiments were determined after heating in an oven at 100^{o}C for 16 h (*Tagetes*, *Primula* and *Lobelia*) and for 40 h (lupin).

Results and discussion

Lupin

There was a significant effect of microwaving on lupin seed germination, with a reduction in percentage germination at microwave durations of 120 sec or more (Table 4.1). There was a general trend for seed to be cleaner with increasing microwave durations but only the sodium hypochlorite treatment significantly increased the percentage of clean seed compared to the untreated control. Longer treatment durations to achieve better pathogen kill would not be suitable, due to deleterious effects on seed germination. It was not possible to determine the effect of microwaving on the incidence of *Colletotrichum acutatum* because infested seed could not be sourced.

Table 4.1: Effect of microwaving on germination of lupin seed and incidence of microbial contamination, 14 days after treatment

Treatment	Mean % normal seed germination		Mean % clean seed	
1. Nil	73.3	(4.89)	56.7	(5.73)
2. 30 sec	66.7	(5.21)	60.7	(5.64)
3. 60 sec	68.0	(5.16)	70.7	(5.26)
4. 120 sec	18.7	(4.31)	66.7	(5.45)
5. 240 sec	0.0	(0.00)	72.0	(5.19)
6. 1% sodium hypochlorite	62.7	(0.05)	95.3	(2.43)
F. probability	<0.001		0.002	

Standard errors in parentheses

Tagetes

There was no effect of microwaving on the germination of *Tagetes* seeds at the durations tested (Table 4.2). There was a significant effect of treatment on the percentage of clean seed but this was due to higher levels of clean seed from the sodium hypochlorite treatment, rather than the microwave treatments which were no different to the control. There was no effect of microwaving on incidence of the target pathogen, *Alternaria* spp.

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Table 4.2: Effect of microwaving on germination of *Tagetes* seed, and incidence of *Alternaria* sp. and microbial contamination, 14 days after treatment

Treatment	% normal seed germination*		% seeds with *Alternaria* sp.		% clean seed	
1. Nil	56.7	(9.66)	8.0	(2.84)	2.7	(1.65)
2. 30 sec	67.3	(9.15)	8.0	(2.84)	2.0	(1.43)
3. 60 sec	75.3	(8.41)	13.3	(3.56)	0.0	(0.00)
4. 120 sec	60.0	(9.55)	3.3	(1.88)	0.0	(0.00)
5. 240 sec	58.0	(9.63)	13.3	(3.56)	0.7	(0.83)
6. 1% sodium hypochlorite	75.3	(8.41)	14.0	(3.63)	22.0	(4.24)
F. probability	0.538		0.142		<0.001	

Standard errors in parentheses

Primula

Microwave treatments of 120 sec or less had no effect on *Primula* seed germination. There was a trend for seed to be cleaner with increasing microwave duration. Microwaving for 240 sec gave significantly cleaner seed than the untreated control, but there was a deleterious effect on germination with this treatment. Sodium hypochlorite treatment gave significantly cleaner seed than the untreated control, without affecting seed germination.

Table 4.3: Effect of microwaving on germination of *Primula* seed, and incidence of microbial contamination, 7 days after treatment

Treatment	% normal seed germination		% clean seed	
1. Nil	88.0	(3.01)	8.0	(3.93)
2. 30 sec	80.0	(3.71)	10.0	(4.35)
3. 60 sec	86.7	(3.15)	16.7	(5.40)
4. 120 sec	77.3	(3.88)	18.7	(5.65)
5. 240 sec	0.7	(0.75)	36.0	(6.97)
6. 1% sodium hypochlorite	81.3	(3.61)	29.3	(6.60)
F. probability	<0.001		0.032	

Standard errors in parentheses

Lobelia

There was a significant effect of microwaving on *Lobelia* seed germination with an increase in germination at microwave durations of 60 and 240 sec, but not at 120 sec (Table 4.4). The level of *Alternaria* infestation on seed was 1% and this was significantly reduced by

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microwave treatment for 120 sec and by the sodium hypochlorite treatment. However, microwave treatment for 240 sec did not reduce *Alternaria* infestation of seed. There was no effect of microwaving on the percentage clean seed.

Table 4.4: Effect of microwaving on germination of *Lobelia* seed, and incidence of *Alternaria* sp. and microbial contamination, 14 days after treatment

Treatment	% normal seed germination		% seeds with *Alternaria* sp.		% clean seed	
1. Nil	41.3	(4.7)	1.0	(0.3)	97.7	(8.9)
2. 60 sec	57.3	(4.7)	0.7	(0.4)	94.7	(18.8)
3. 120 sec	43.3	(4.7)	<0.1	(0.0)	96.0	(16.4)
4. 240 sec	58.0	(4.7)	1.3	(0.5)	95.4	(17.5)
5. 1% sodium hypochlorite	52.7	(4.8)	0.3	(0.2)	95.0	(12.8)
F. probability	0.032		0.032		0.425	

Standard errors in parentheses

Seeds of the four species used varied greatly in size (Table 4.5). *Primula* and lupin seed had dry weights above 90% while *Lobelia* had the lowest dry weight (67%). The adverse effect of microwave treatment on germination of *Primula* and lupin, and not *Lobelia* and *Tagetes*, was not obviously related to either seed size or dry weight.

Table 4.5: Effect of seed size and species on dry weight

Species	Fresh weight of 1,000 seeds (g)	% dry weight	Effect of microwave treatment on germination
Lobelia	0.038	67	Nil
Primula	0.750	93	At high durations (240 sec)
Tagetes	3.000	82	Nil
Lupin	22.000	91	At low durations (120 sec)

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5. Effects of aerated steam treatments on seed-borne fungi

Introduction

The objectives of the study were:

1. To run range-finder experiments with batches of seed of three sizes to determine seed viability following steam-air sterilisation treatments.

2. To utilise the information generated in objective 1 to carry out steam-air treatments and determine the effect on microbial contamination and germination of seed of three selected ornamental species, representing three different seed sizes.

3. To utilise the information generated in objectives 1and 2 to determine the effect of steam-air treatments on the incidence of target pathogens on seed of the selected ornamental species.

Materials and methods

A series of range finder experiments were carried out on batches of seed of lupin, *Tagetes* and *Primula*, representing three different seed sizes.

The temperature and duration parameters on the steam air steriliser were set based on previous tests (for a commercial seedhouse) where 20 min at 50^{o}C was found to be optimum for *Lobelia* seed (Table 5.1). During treatment, it was necessary to leave two empty seed trays and then place 100 seed of each species in the next three trays, which were labelled with the seed type and treatment number. Following treatment each seed batch was retained in labelled paper envelopes. Seed was left to cool before setting up germination or agar plate tests.

Table 5.1: Treatment temperatures and durations under investigation

10 minutes	20 minutes	30 minutes
T1 50°C	T5 45°C	T9 40°C
T2 55°C	T6 50°C	T10 45°C
T3 60°C	T7 55°C	T11 50°C
T4 65°C	T8 60°C	T12 55°C

Due to the large number of tests, single samples were treated (100 seed each) and subjected to germination tests following treatment. Seed batches were assessed at 7 and 14

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days using the method described in Section 4. Batches of untreated seed were germinated alongside the treated samples for comparison.

Following the initial range-finder experiments, the three temperature and duration combinations which resulted in the highest level of germination for each seed type were subsequently tested in a main experiment. Treated seed batches from the main experiment were compared with seed that had either received no treatment (untreated control) or received a standard surface sterilisation treatment in sodium hypochlorite (1% available chlorine) for 5 min, giving five treatments in total. Seeds for the untreated control received no steam-air or surface sterilisation treatment. For each treatment, there were three replicate samples of 100 seeds each.

After treatment of each replicate seed sample, 50 seeds were plated onto agar using aseptic procedures. The incidence of fungal and bacterial growth was assessed from each seed, 7 and 14 days after plating. In addition, 50 seeds per replicate were used for germination tests (Fig. 5.1).

Fig 5.1: *Tagetes* seedlings germinating following steam-air treatment

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Results and discussion

The range finder tests provided some interesting and useful data. The germination rates for the treated lupin seed appeared to be affected to a greater degree by the treatment than the other seed types (*Tagetes* and *Primula*) and this may be linked to the size and water content of the seed, and/or age/vigour of the seed batch (Table 5.2, Fig 5.2). In general, better germination was observed following lower temperature treatments. The germination rates of treated *Tagetes* seed remained high for the majority of treatments, only being severely affected at temperatures of 60 and 65°C. *Primula* seed appeared able to withstand temperatures up to 50°C at short treatment durations, with little effect on germination rates. Results for this species were rather variable at longer treatment durations.

During the treatment process some difficulties were experienced in maintaining the set temperature for the duration of the treatment. Tiny tag data loggers were used to record the temperature within the treatment chamber during the main experiment. Downloaded data indicated that the average (mean) temperature recorded during each treatment was generally within 1-2°C of the set temperature.

The data from the main experiment for the steam air study is shown in Tables 5.3 – 5.5.

<u>Lupin</u>

The germination rates for the seed at each temperature and duration combination were high, as would be expected based on the range finder experiments (Table 5.3). Percentage normal germination in the steam air treatments was not significantly different from the untreated control. Seed germination was significantly reduced in the sodium hypochlorite treatment.

Steam air treatments did not increase the incidence of 'clean' lupin seed in comparison with the untreated control; there was actually a significant reduction in the incidence of clean seed following treatment at 40^{o}C for 30 min. Sodium hypochlorite significantly increased the incidence of clean lupin seed. No incidence of the target pathogen (*Colletotrichum acutatum*) was recorded on any of the seed during this test. A range of other contaminating organisms were detected on the seed (Table 5.3)

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Table 5.2: Effect of steam air treatment on germination of three ornamental seed species (range finder tests)

Treatment number	Treatment temperature and duration	Percentage seeds in each category after 14 days											
		Lupin				*Tagetes*				*Primula*			
		Normal	**Abnormal**	**Fresh**	**Dead**	**Normal**	**Abnormal**	**Fresh**	**Dead**	**Normal**	**Abnormal**	**Fresh**	**Dead**
Control	Untreated	98	0	0	1	93	4	0	3	75	0	25	0
1	50°C 10 min	93	3	0	4	98	2	0	0	73	6	13	8
2	55°C 10 min	53	46	0	1	99	0	0	1	61	0	38	1
3	60°C 10 min	34	65	0	1	72	0	0	28	0	1	0	99
4	65°C 10 min	0	49	0	51	0	9	18	73	0	0	0	100
5	45°C 20 min	97	2	0	1	99	0	0	1	69	0	29	2
6	50°C 20 min	94	2	0	4	97	3	0	0	15	0	74	11
7	55°C 20 min	85	15	0	0	91	2	0	7	24	0	65	10
8	60°C 20 min	0	24	0	76	0	22	0	78	0	0	0	100
9	40°C 30 min	73	26	0	1	99	1	0	0	85	0	15	0
10	45°C 30 min	82	18	0	0	100	0	0	0	81	0	17	2
11	50°C 30 min	52	43	0	5	88	12	0	0	95	1	0	4
12	55°C 30 min	88	9	0	1	90.82	6.12	0	3.06	2	0	93	5

Highlighted areas show the temperatures and durations taken forward for the main experiment.

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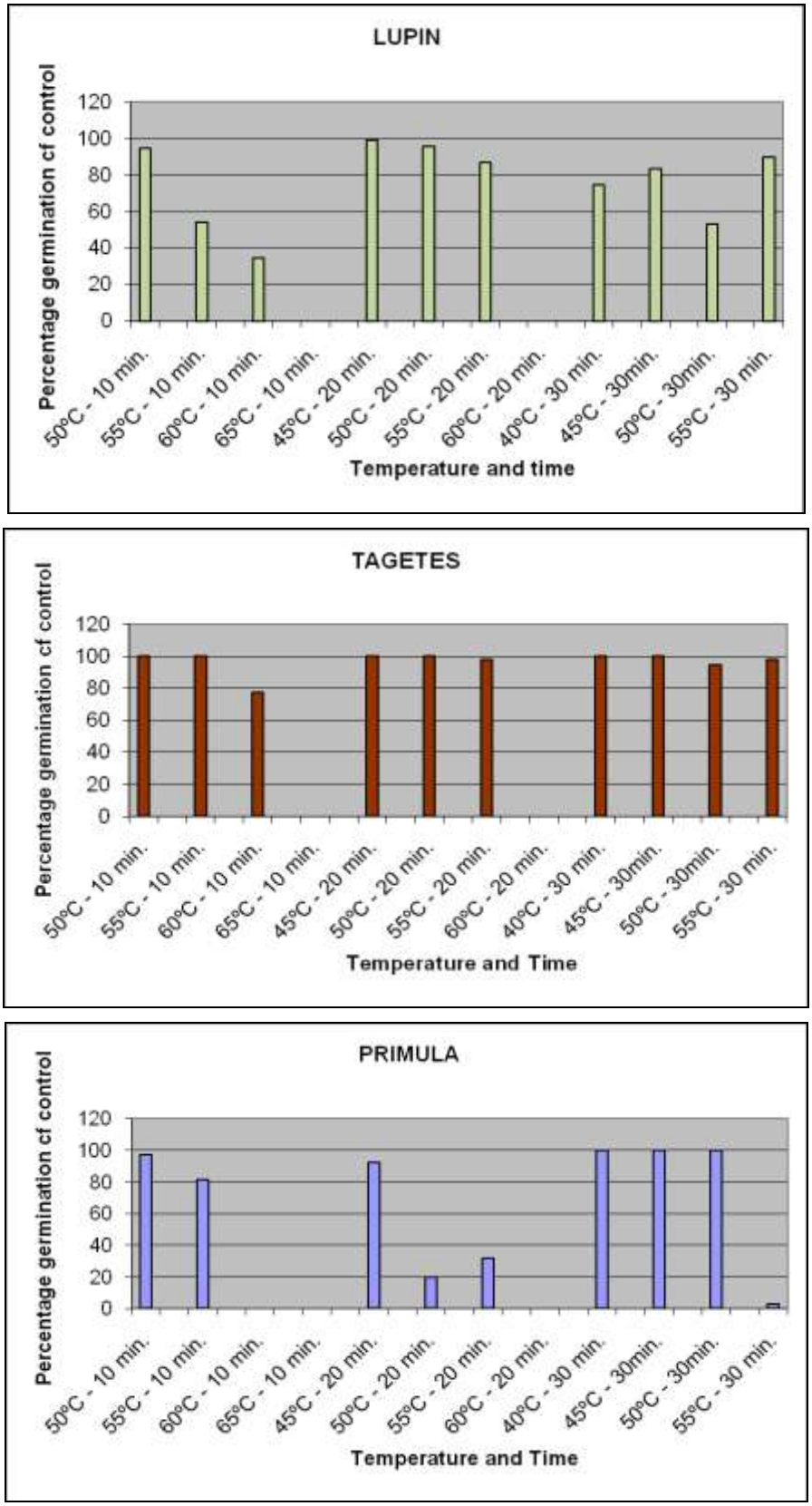

Figure 5.2: Showing the percentage of seed germinated at each temperature and duration (steam air treatment) as a percentage of the untreated control

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Table 5.3: Effect of steam air treatment on lupin seed germination and microbial contamination

Treatment	Mean percentage seed (3 reps) after 14 days						
	Germination tests				Seed contaminant tests		
	% Normal	% Abnormal	% Fresh	% Dead	% Clean seed	% seed with target pathogen	Primary contaminants
Control	98.0 (1.34)	2.0	0.0	0.0	72.0 (3.84)	0	*Penicillium, Aspergillus, Alternaria*
Surface disinfected	82.7 (3.63)	6.7	0.0	10.6	87.3 (3.37)	0	*Fusarium, Penicillium*
50°C, 10 min	97.3 (1.55)	2.7	0.0	0.0	66.7 (4.03)	0	*Penicillium, Cladosporium, Mucor*
45°C, 20 min	96.7 (1.72)	2.7	0.0	0.6	72.7 (3.81)	0	*Penicillium, Cladosporium, Trichoderma*
40°C, 30 min	96.7 (1.72)	2.7	0.0	0.6	51.3 (4.27)	0	*Penicillium*
F. probability	0.007				0.005		

Standard errors in parentheses.

Tagetes

The germination rates of the steam air treated *Tagetes* seed were similar to those seen in the untreated and surface disinfected seed for the two lower temperature/duration treatments. However, a significant reduction in germination was observed at the higher temperature (55°C) treatment which was contrary to the effect seen during the range finder experiment when a germination rate of 99% was recorded. Tiny tag data recorded during this treatment application shows that although the average temperature was slightly under 55°C for each replicate, the temperature peaked at 56 and 57°C within each replicate for very short periods and this may have been enough to reduce the germination rate.

A comparison of the percentage of 'clean' seed indicates that steam air treatment at 55°C for 10 mins significantly reduced the amount of surface contamination on the seed compared with that observed on the untreated and surface disinfected seed, whilst the other steam air treatments were ineffective. Some evidence of the target pathogen (*Alternaria* sp.) was

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observed on all the treated seed batches, with the exception of the 45°C for 20 min treatment, although it was markedly reduced in all the treated seed (Table 5.4).

Table 5.4: Effect of steam air treatment on *Tagetes* seed germination and microbial contamination

Treatment	Mean percentage seed (3 reps) after 14 days						
	Germination tests				Seed contaminant tests		
	% Normal	% Abnormal	% Fresh	% Dead	% Clean seed	% seed with target pathogen	Primary contaminants
Control	98.0 (2.35)	2.0	0.0	0.0	55.3 (5.36)	4.7	*Fusarium, Cladosporium, Bacteria, Penicillium*
Surface disinfected	98.7 (1.93)	1.3	0.0	0.0	44.3 (3.42)	1.3	*Fusarium, Penicillium & Phomopsis*
55°C, 10 min	64.0 (8.07)	13.3	0.0	22.7	80.7 (4.26)	1.3	*Cladosporium, Penicillium*
45°C, 20 min	99.3 (1.35)	0.7	0.0	0.0	66.5 (5.24)	0.0	*Fusarium, Cladosporium, Trichoderma, Mucor*
40°C, 30 min	98.7 (1.93)	1.3	0.0	0.0	48.7 (5.39)	2.7	*Fusarium, Penicillium, Cladosporium, Trichoderma, Mucor,*
F. probability	0.003				<0.001		

Standard errors in parentheses

The results of the main-experiment steam-air testing on *Primula* seed are shown in Table 5.5. Some severe anomalies in the germination rates were seen in this experiment compared with those observed in the range-finder experiment. In this experiment, little or no germination was observed following treatment at 55°C for 10 minutes and 50°C for 30 minutes compared with 61% and 95% respectively in the range-finder test. The reasons for these differences are not clear; however temperature fluctuations during the experiment cannot be discounted. The germination rates for the 45°C/20 minute treatment and the sodium hypochlorite treatment were not significantly different from the untreated seed.

There was a trend for a significant increase in clean seed following steam air treatment of 55°C but this was not statistically significant. Treatment with sodium hypochlorite completely

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eliminated seed contamination. The target pathogen (*Botrytis cinerea*) was detected at low levels (<3%) on seed from each temperature/duration treatment, although not at all in either the untreated or surface sterilised seed.

In conclusion, aerated-steam treatment generally had little detrimental effect on germination rates at temperatures up to 50°C for durations up to 30 min. However, susceptibility to treatment varied with seed type, and also between different runs of the experiment, due probably to temperature variation in the equipment used (1-2^{o}C deviation from the set temperature). Some slight reduction in surface contamination was observed following treatment of *Tagetes* and *Primula* seed, whilst no benefits were observed in lupin seed. Overall this treatment appeared to be less effective than surface disinfection with sodium hypochlorite.

Given the complexity of the equipment trialled it is concluded that in its current form, steam-air seed treatment is not an economically viable option compared to other seed disinfection treatments.

Table 5.5: Effect of steam air treatment on *Primula* seed germination and microbial contamination

Treatment	Mean percentage seed (3 reps) after 14 days						
	Germination tests				Seed contaminant tests		
	% Normal	% Abnormal	% Fresh	% Dead	% Clean seed	% seed with target pathogen	Primary contaminants
Control	76.0 (4.45)	0.0	20.7	3.3	71.3 (8.27)	0	*Cladosporium, Fusarium, Mucor & Penicillium*
Surface disinfected	79.3 (4.22)	0.0	20.7	0.0	100.0 (0.00)	0	*None*
55°C, 10 min	1.4 (1.20)	0.0	21.3	77.3	90.0 (5.49)	2.6	*Cladosporium & Bacteria*
45°C, 20 min	72.0 (5.73)	0.0	17.0	11.0	77.3 (7.66)	0.6	*Cladosporium, Alternaria, Bacteria, Trichoderma, Penicillium*
50°C, 30 min	0.0 (0.00)	0.0	13.3	86.7	64.0 (8.78)	0.6	*Cladosporium, Bacteria, Alternaria, Penicillium*
F. probability	<0.001				0.015		

Standard errors in parentheses

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6. Effects of fungicides on seed-borne fungi

Introduction

The aim of the experiment was to determine the effect of a new seed treatment formulation of iprodione (Rovral AquaFlo) on the incidence of *Alternaria* species on *Lobelia* seed. A batch of *Lobelia* naturally infested with *Alternaria* was provided by a commercial seedhouse.

Materials and methods

Treatments

Untreated control

1. Sodium hypochlorite (5 mins in 1% available chlorine)
2. Rovral AquaFlo (50% iprodione)
3. Rovral AquaFlo followed by sodium hypochlorite (as above)

The effects of treatments on microbial contamination were tested for treatments 1-4; effects on seed germination were tested for treatments 1-3.

Experiment design and statistical analyses

Three replicate samples of seed were used for each treatment. Seed germination tests were done on 50 seeds per replicate (150 seeds per treatment). For evaluation of microbial contamination there were 100 seeds per replicate (300 seeds per treatment).

The effects of treatments on seed germination and the incidence of microbial contamination were determined by generalised linear models in Genstat.

Seed treatment

20 g *Lobelia* seed to be treated with Rovral AquaFlo was sent to HRI (University of Warwick). The product was applied at a rate of 5 L per tonne seed (0.1 ml per 20 g seed). Ciba 'Unisperse Blue B-5' dye was also added to the fungicide before treatment at a rate of 2.5%, according to manufacturer's instructions. The treatment was applied as a fluidised-bed film coating (A. Jukes, pers. comm.). The rates used at the time of treatment (above) was according to available SOLAs for vegetable seed; after the experiment was completed, an

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on-label approval for treatment of ornamental seed (including *Lobelia*) has been issued, using a dip in Rovral AquaFlo at 0.1 L/100 L water.

Seeds to be surface disinfected were immersed for 5 min in sodium hypochlorite solution (1% available chlorine). Excess liquid was decanted off through muslin, then seeds were placed in a laminar flow cabinet to dry.

Seed germination tests

Methods were as for microwave treatment experiments.

Incidence of microbial contamination

Methods were as for microwave treatment experiments.

Results and discussion

Germination was low (49% overall) and was not significantly affected by seed treatment. Although both the sodium hypochlorite and Rovral AquaFlo seed treatments appeared to reduce infection with *Alternaria* sp., differences were not significant. The combined sodium hypochlorite and Rovral AquaFlo seed treatment significantly increased the proportion of clean seed.

Table 6.1: Effect of Rovral Aquaflo fungicide on germination of *Lobelia* seed, and incidence of *Alternaria* sp. and microbial contamination, 14 days after treatment

Treatment	**% normal seed germination**		**% seeds with *Alternaria* sp.**		**% clean seeds**	
1. Nil	41.3	(4.7)	1.0	(0.3)	97.7	(0.7)
2. 1% sodium hypochlorite	52.7	(4.8)	0.3	(0.2)	95.0	(1.1)
3. Rovral AquaFlo	52.0	(4.8)	0.3	(0.2)	98.0	(0.7)
4. Rovral AquaFlo + 1% sodium hypochlorite	ND	ND	0.3	(0.2)	99.3	(0.4)
F. probability	0.173		0.132		0.002	

Standard errors in parentheses; ND – not determined.

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Conclusions

2007 (Year 1)

- Surface sterilisation with sodium hypochlorite (1% available chlorine) for 5 minutes, reduced contamination due to saprophytic fungi and non-pathogenic bacteria in all but four seed lots of 29 tested.

- *Botrytis cinerea, Alternaria* species and a *Pythium* species were the only suspect fungal pathogens recovered, being isolated from ten, five, one and one seed lot(s), respectively.

- For some crops (*Pelargonium*, lupin, pansy, *Phlox* and *Zinnia*), *B. cinerea* was isolated from surface sterilised seed, suggesting more deep-seated seed infection rather than surface contamination. The highest incidence of infection was found on surface sterilised seed of pansy (7% seeds infected).

- *Alternaria* species were isolated from four of the six crops for which they were listed as potential seed-borne pathogens. The highest incidence was found on *Zinnia* (up to 40% infection). A pathogenicity test confirmed that *Alternaria cheiranthi* recovered from wallflower seed was pathogenic to wallflower.

- Pathogenic bacterial species (*Pseudomonas* and *Xanthomonas* species) were not recovered from any of the seed lots.

- A sclerotial-forming fungus *Streptobotrys streptothrix* (anamorph: *Botrytis streptothrix*) that had not previously recorded in the UK was isolated from *Lobelia* seed. The fungus was weakly pathogenic to *Lobelia*.

2008 (Year 2)

- Out of 25 commercial seed lots of 15 ornamental species tested by plating onto agar, the suspect pathogens *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) were recovered.

- None of the pathogenicity tests carried out using suspect pathogens isolated from seed in year 2 were positive.

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2009 (Year 3)

- Out of 32 commercial seed lots of 18 ornamental species tested by plating onto agar, the suspected pathogens *Alternaria* spp. (on *Aquilegia*, *Lobelia*, lupin, *Rudbeckia*, *Verbena* and *Zinnia*), *Botrytis cinerea* (on *Primula* and *Zinnia*), *Fusarium* spp. (on *Cyclamen* and stock) and *Phoma* sp. (on *Lychnis*) were recovered.

- Pathogenicity of fungi and bacteria recovered from seed was confirmed for unidentified bacteria on *Antirrhinum*, *Alternaria* sp. on *Cineraria*, lupin, phlox and *Tagetes*, *Alternaria* sp. and *Fusarium* sp. on *Lobelia*, *Alternaria* sp. and *B. cinerea* on *Zinnia*, *B. cinerea* on *Primula* and *F. oxysporum* on *Cyclamen*.

- Microwave treatment up to 60 sec duration had no adverse effect on germination of *Lobelia*, lupin, *Primula* or *Tagetes* seed but more extended treatments greatly reduced germination of lupin and *Primula*.

- Microwave treatment of lupin and *Primula* seed increased the proportion of seeds with no fungal or bacterial growth but only at durations that reduced germination.

- Rovral AquaFlo at the rate used in this work did not reduce *Alternaria* on *Lobelia*.

- Treatment of seed with sodium hypochlorite (5 minute dip in 1% solution) increased the proportions of clean seed of lupin, *Tagetes* and *Primula* without adversely affecting seed germination.

- Aerated-steam treatment generally had little detrimental effect on germination rates at temperatures up to 50°C for durations up to 30 min. However, susceptibility to treatment varied with seed type, and also between different runs of the experiment, due probably to temperature variation in the equipment used (1-2^{o}C deviation from the set temperature). Some slight reduction in surface contamination was observed following treatment of *Tagetes* and *Primula* seed, whilst no benefits were observed in lupin seed. Overall this treatment appeared to be less effective than surface disinfection with sodium hypochlorite.

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Technology transfer

Project review meeting

Stratford on Avon, 27 May 2008

Articles

O'Neill, T.M. (2007). Health check on seeds. *HDC News* **137:**24-25

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Factsheet

Seed-borne diseases of ornamentals: prevalence and control (in preparation).

Presentations

- Protected ornamentals: detection, prevalence and control of seed-borne diseases. HDC/ BPOA/BOPP Technical Seminar, Northampton, 25 June 2008 (Tim O'Neill).
- Options for control of seed-borne diseases. HDC/BPOA Technical Seminar at Warwick HRI, 18 February 2009 (Erika Wedgwood).

Project progress meetings

STC – 15 February 2006

STC – 6 February 2007

ADAS - 25 May 2008

Conference call – 15 May 2009

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APPENDICES

Appendix 1 Detail of STC pathogenicity tests

Year 2

Bacterial isolates on Aquilegia

A total of 4 bacterial isolates were tested on Aquilegia seedlings which were raised from seed. A turbid suspension of each of the unidentified bacteria was applied as droplets to the foliage of seedlings both with and without damage. The inoculated seedlings were then incubated in seedling germinators alongside untreated damaged and undamaged control seedlings. None of the isolates tested resulted in any symptom or lesions developing.

Alternaria on Verbena

Three morphologically different isolates of *Alternaria* were tested on Verbena seedlings. A spore suspension was sprayed onto damaged and undamaged seedlings prior to incubation. No symptoms consistent with an *Alternaria* infection were observed on the seedlings and the isolates were deemed non-pathogenic.

Year 3

Bacterial isolates

Unidentified bacterial isolates collected from Antirrhinum, Pelargonium and Wallflower were tested on seedlings of the same host. Droplets of each of the suspended bacterial isolates were placed on the leaves of the undamaged plants, other plants were stab inoculated into the stem with the bacterial isolate. All inoculated plants were incubated in plastic bags alongside uninoculated control plants. Tests on Pelargonium and Wallflower were negative, 1 isolate of bacteria produced symptoms on the damaged Antirrhinum seedlings and was considered to be pathogenic.

Cineraria

Two *Alternaria* sp. isolates collected from the seed testing on Cineraria were tested on young seedlings. The isolates were inoculated onto seedling leaves on small agar pieces with and

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without damage to the leaves. Although both isolates resulted in the formation of lesions on both the undamaged and damaged leaves (see Figure), close examination of the spore type showed neither isolate to conform to *A. cinerariae* or *A. denisii*.

The pathogenicity of two isolates of bacteria and a *Ulocladium* sp. was also tested. None were found to be pathogenic.

Lobelia

An isolate of *Alternaria* and 2 *Fusarium* isolates were tested on young seedlings. The Alternaria sp. produced lesions on both the damaged and undamaged seedlings and was considered pathogenic. Colony and spore morphology was considered consistent with *A. tenuissima.* One of the *Fusarium* sp. isolated resulted in severe lesions on both the damaged and undamaged leaves resulting in complete plant collapse.

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Lupin

An isolate of *Alternaria* which was detected on 6% of the non-surface sterilised seed was tested on young seedlings and found to be pathogenic on both damaged and undamaged seedlings.

Phlox

The pathogenicity of several isolates of *Alternaria* was tested on young Phlox seedlings. All were found to be pathogenic to some degree, resulting in the formation of lesions on the inoculated leaves.

Primula

An isolate of *Botrytis* found on 2.6% of the non-surface sterilised Primula seed was found to be pathogenic on young seedlings.

Control (undamaged & damaged)

Botrytis inoculated (undamaged & damaged)

Zinnia

Five isolates of *Alternaria* sp. collected from both surface sterilised and non-sterilised seed were tested. All were found to be pathogenic to some degree. However, the morphology of the colonies and spores was not consistent with *A. zinniae*.

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