Contract report for Horticultural Development Council

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

PC 252

June 2007

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

Tel: 01732 848 383
Fax: 01732 848 498

Project title:	Protected ornamentals: detection, prevalence and control of seed-borne diseases
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Project leader:	Dr T M O'Neill ADAS UK Ltd Mepal, Ely Cambs CB6 2BA
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Key workers:	Dr K Green, Ms A Shepherd & Ms K Wynn, ADAS Dr G M McPherson & Ms C Lambourne, STC Mr S Coutts – Consultant to project
Location:	Research facilities at ADAS Arthur Rickwood and STC; UK nurseries
Project co-ordinator:	Ms F Richardson Coletta & Tyson
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The results and conclusions in this report are based on experimental work conducted over one year. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

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AUTHENTICATION

I declare that this work was done under my supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

Dr T M O'Neill
Principal Research Scientist
ADAS Arthur Rickwood

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

Ms C Lambourne
Plant Pathologist
Stockbridge Technology Centre

Signature …….. Date

Report authorised by:

Dr W E Parker
Horticulture Sector Manager
ADAS Wolverhampton

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

Dr G M McPherson
Plant Pathologist
Stockbridge Technology Centre

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

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GROWER SUMMARY

Headline

This project has compiled a comprehensive listing of bedding and pot plant diseases that can originate from the use of infected seed identified main four pathogens in tests on commercial seed lots. For species commonly affected by seed-borne pathogens, growers should examine plants at an early growth stage and take prompt action if disease is found.

Background and expected deliverables

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

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

Summary of the project and main conclusions

Seed-borne diseases of protected bedding and pot plant species

Lists of confirmed and suspected seed-borne diseases of common bedding and pot plant species grown in the UK have been 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 diseases; *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 pansy seed is being investigated in a separate project (PC 231). The full lists are available in the Science Section of this report; a condensed list summarising confirmed and suspected (marked as ***) seed-borne diseases of key protected crops grown in the UK is shown in Table 1.

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

Ornamental species and pathogen	Disease	Pathogen reported in the UK*	Recent occurrence of the disease **
Alyssum			
Alternaria sp.	Leaf spot	Yes	Uncommon
Stemphylium botryosum	Leaf spot	Yes	Uncommon
Sclerotinia sclerotiorum	Tissue rot	Yes	Quite common
Pseudomonas sp.***	Bacterial leaf spot	Yes	Quite common
Antirrhinum majus (snapdragon)			
Alternaria alternate	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	Rare
Pleospora herbarum		Yes	
Pseudomonas syringae pv. *antirrhini*	Bacterial leaf spot	Yes	Occasional
Puccinia antirrhini	Rust	Yes	Quite common
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	Bacterial wilt	Yes	Quite common
Cyclamen persicum (cyclamen)			
Botrytis cinerea	Grey mould	Yes	Common
Colletotrichum gloeosporioides	Anthracnose	Yes	Uncommon
Fusarium oxysporum f. sp. c*yclaminis*	Wilt	Yes	Quite common
Potato virus X			

Ornamental species and pathogen	Disease	Pathogen reported in the UK*	Recent occurrence of the disease **
Ramularia cyclaminicola	Leaf spot/stunt		
Septoria cyclaminis	Leaf spot		
Tomato mosaic virus			
Impatiens spp. (Busy Lizzie or snapweed)			
Alternaria zinniae		Yes	
Phyllosticta impatientis			
Plasmopora obducens	Downy mildew	Yes	Widespread in 2003 and 2007
Rhizoctonia solani			
Lobelia			
Alternaria alternate	Leaf spot and stem rot	Yes	Common
Lupinus spp. (lupin)			
Bean yellow mosaic virus		Yes	
Botrytis cinerea	Grey mould	Yes	
Colletotrichum acutatum	Anthracnose	Yes	Common
Cucumber mosaic virus			
Diaporthe woodii	Phomopsis stem blight		
Erwinia sp.			
Fusarium oxysporum	Wilt		
Gibberella avenacea			
Glomerella cingulata	Anthracnose	Yes	
Pea mosaic virus			
Peanut mottle virus			
Peanut stunt virus			
Pleiochaeta setosa	Brown spot	Yes	
Pseudomonas spp.			
Sclerotinia sclerotiorum		Yes	
Stemphylium sp	Leaf spot		
Verticillium albo-atrum	Wilt	Yes	
Verticillium sp.	Wilt		
Nicotiana spp. (tobacco)			
Alternaria alternate			
Alternaria longipes			
Alternaria zinniae		Yes	
Arabis mosaic virus			
Arracacha virus A			
Artichoke yellow ringspot virus			
Asparagus virus 2			
Botrytis cinerea	Grey mould	Yes	
Cassava green mottle virus			
Cercospora nicotianae	Green spot		
Cherry leaf roll virus			
Colletotrichum tabacum	Anthracnose		
Corynebacterium fascians			
Eggplant mosaic virus			
Erwinia carotovora ssp. *carotovora*	Tobacco hollow stalk		
Erwinia spp.			
Nicotiana velutina mosaic virus			
Pelargonium zonate spot virus			

Ornamental species and pathogen	Disease	Pathogen reported in the UK*	Recent occurrence of the disease **
Peronospora tabacina	Downy mildew	Yes	Quite common
Potato (Andean) latent virus			
Potato virus U			
Pseudomonas aeruginosa	Phillipine leaf spot		
Pseudomonas syringae pv. *mellea*	Wisconsin leaf spot		
Pseudomonas syringae pv. *tabaci*	Wildfire		
Rubus Chinese seed-borne virus			
Spinach latent virus			
Tobacco ascending necrosis virus			
Tobacco etch virus			
Tobacco mosaic virus			
Tobacco ringspot virus			
Tobacco streak virus			
Tobacco black ring virus			
Tomato ringspot virus			
Xanthomonas heterocea	Bacterial leaf spot		
Pelargonium sp. (pelargonium, geranium)			
Pseudomonas sp.	Bacterial leaf spot	Yes	Uncommon
Tobacco ringspot virus			
Tomato ringspot virus			
Phlox drummondii (phlox)			
Alternaria sp.			
Cochliobolus lunatus			
Rhizoctonia solani	Damping off		
Septoria drummondii	Leaf spot	Yes	Quite common
Primula spp. (primrose, polyanthus)			
Botrytis cinerea	Grey mould	Yes	Common
Phyllosticta primulicola	Leaf spot		
Pseudomonas syringae pv. *Primulicola*	Bacterial leaf spot		
*Ramularia agrestis****	Leaf spot	Yes	Common
Senecio cruentus (cineraria)			
Alternaria cinerariae	Leaf spot	Yes	Quite common
Botrytis cinerea	Grey mould	Yes	
Erysiphe cichoracearum	Powdery mildew	Yes	
Cineraria mosaic virus			
Viola (pansy, violet)			
Cherry leaf roll virus			
Colletotrichum violae-tricoloris	Anthracnose		
Mycocentrospora acerina	Halo blight	Yes	Uncommon
Phoma sp.			
Ramularia lacteal	Leaf spot	Yes	Common
Rhizoctonia solani		Yes	
Sphaceloma violae	Scab		
Tobacco rattle virus			
Urocystis violae	Smut		
*Ramularia agrestis****	Leaf spot	Yes	
Zinnia elegans (zinnia)			

Ornamental species and pathogen	Disease	Pathogen reported in the UK*	Recent occurrence of the disease **
Alternaria zinniae	Leaf spot/blight	Yes	Common
Asparagus virus 2			
Botrytis cinerea	Grey mould	Yes	Common
Colletotrichum acutatum			
Erysiphe cichoracearum	Powdery mildew		
Glomerella cingulata			
Phyllosticta sp.			
Rhizoctonia solani	Damping off		
Tobacco ringspot virus			
Tomato black ring virus			
Xanthomonas campestris pv. *zinniae*			

Notes:
More detailed crop/pathogen lists are provided in the Science Section of the Year 1 Annual Report for PC 252 (Tables 2, 3 and 4).

*****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.
***Suspected as a seed-borne pathogen, but not confirmed.

Prevalence of seed-borne pathogens in 2006

There are various ways in which seed can transmit plant pathogens:

- Contamination – the pathogen is carried with the seed but is not attached to it (e.g. sclerotia of *Sclerotinia sclerotiorum).*
- Superficial infection – the pathogen is located on the outside of the seed or fruit coat.
- Internal infection – 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.

Standard methods were devised and used to test 29 commercial seed lots from 18 ornamental species for key seed-borne fungal and bacterial pathogens. The seed was obtained from UK plant propagators and was supplied in unopened packets. In summary:

- No sclerotia of *S. sclerotiorum* were found in any of the seed lots.
- Surface sterilisation with sodium hypochlorite (1% available chlorine) for 5 minutes, reduced contamination by fungi and non-pathogenic bacteria in all but four seed lots.
- Of the potential pathogens sought on seed, *Botrytis cinerea, Alternaria* species, *Colletotrichum acutatum* and *Pythium* species were the only fungal pathogens recovered, being isolated from ten, five, one and one seed lot(s), respectively.
- *Botrytis cinerea* was isolated from seed of nine species, including five from which it has not previously been reported (lobelia, pelargonium, phlox, viola and wallflower).
- For some crops (pelargonium, lupin, pansy, phlox and zinnia), *B. cinerea* was isolated from surface sterilised seed, suggesting 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 (zinnia, wallflower, aquilegia and cineraria). The highest incidence was found on zinnia (up to 40% infection); pathogenicity of the recovered *Alternaria* species to zinnia is to be tested. A pathogenicity test confirmed that *Alternaria cheiranthi* recovered from wallflower seed was pathogenic to wallflower.
- Pathogenic bacterial species (*Pseudomonas* and *Xanthomonas* species) have not as yet been recovered from any of the seed lots.
- A sclerotial-forming fungus *Streptobotrys streptothrix* (asexual stage: *Botrytis streptothrix*) that had not previously recorded in the UK was isolated from lobelia seed. Pathogenicity to lobelia is currently being tested.

Financial benefits

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

Action points for growers

- Growers should be aware of the potential seed-borne origin of key diseases of important pot and bedding plant species as detailed in Table 1.

- For species commonly affected by seed-borne pathogens, examine plants for disease at an early growth stage. Take action promptly to control any diseases found (e.g. lupin anthracnose).

SCIENCE SECTION

Introduction

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

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

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

Government, retailers and consumers demand sustainable production with minimal use of pesticides. When a disease outbreak on a nursery originates on seed, the most efficient and effective method of control, requiring minimal use of pesticides, is by an appropriate seed treatment. In some instances physical treatments (e.g. heat, hot air) are effective. Where a non-chemical method of control is unavailable, a single chemical treatment of the seed may result in satisfactory control, eliminating the need for routine fungicide applications during crop production. In addition to savings on costs, an effective seed treatment has minimal adverse impact on the environment, poses little health risk to nursery staff and helps to avoid fungicide resistance problems, in comparison with a series of fungicide sprays. It is accepted that some seeds are difficult to treat due to the slimy or sticky nature of the seed.

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

List of seed-borne diseases of protected bedding and pot plant species

A list of seed-borne diseases of protected bedding and pot plants species commonly grown in the UK is given in Table 2. This list includes bacterial, fungal, virus and viroid diseases. It was compiled largely from information listed by M.J. Richardson (1990) in 'An Annotated List of Seed-Borne Diseases' published by the International Seed Testing Association (ISTA). New reports of seed-borne diseases published since 1990, and unpublished results of seed tests by the authors or undertaken for independent consultants, are also listed. The list in Table 2 does not discriminate between *transmission*, in which infected seed produces infected plants, and *seed transport*, in which an organism is demonstrated to be present on the seed but has not been shown subsequently to cause infection. Transport by seed may be relatively unimportant in crop production, but it is one way in which a pathogen may be introduced into an area from which it was previously absent.

Crop species in Table 2 are arranged in alphabetical order of the host Latin name. Within each host list the pathogens are listed alphabetically. Where the disease has a common name, this is also given.

The list comprises reports of seed-borne diseases from throughout the world. Where the pathogen is known to occur in the UK, this is indicated. It should be noted that this does not necessarily mean that the pathogen has occurred on the particular host in the UK.

Table 3 gives a list of pathogens that are suspected but not proven as seed-borne pathogens of bedding and pot plant species. Table 4 presents a summary of some key features of selected seed-borne diseases of these crops. The host species selected are those that are currently grown commonly in the UK. The diseases selected are ones known to occur in the UK.

Table 2: List of seed-borne diseases of common bedding and pot plant species grown in the UK

Ornamental species and pathogen	Disease	Pathogen reported in the UK*	Reference**
Ageratum spp.			
Alternaria solani	Early blight	Yes	
Botrytis cinerea	Grey mould	Yes	
Althaea spp. (hollyhock)			
Colletotrichum malvarum	Anthracnose	Yes	
Puccinia heterospora	Rust		
Puccinia malvacearum	Rust		
Alyssum			
Alternaria sp.	Leaf spot	Yes	Maude, pers. comm., 1997
Stemphylium botryosum	Leaf spot	Yes	Maude, pers. comm., 1997
Sclerotinia sclerotiorum	Tissue rot	Yes	Maude, pers. comm., 1997
Antirrhinum majus (snapdragon)			
Alternaria alternate	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	Baker, 1972; Rosser, 1961
Pleospora herbarum		Yes	
Pseudomonas syringae pv. *Antirrhini*	Leaf spot	Yes	Simpson *et al*., 1971
Puccinia antirrhini	Rust	Yes	
Aubrietia spp.			
Alternaria brassicae	Grey leaf spot		
Alternaria brassicicola	Black spot	Yes	
Phoma aubretiae			
Phoma sp.			
Bellis perennis (daisy)			
Phoma bellidis			
Brassica spp. (ornamental cabbage)			
Albugo candida	White blister	Yes	
Alternaria brassicae	Grey leaf spot	Yes	
Alternaria brassicicola	Black spot	Yes	
Alternaria raphani			
Alternaria spp.			
Ascochyta oleracea	Leaf spot		
Botrytis cinerea	Grey mould	Yes	
Cladosporium tenuissimum			

Ornamental species and pathogen	Disease	Pathogen reported in the UK*	Reference**
Erysiphe sp.	Powdery mildew	Yes	
Fusarium oxysporum f. sp. *conglutinans*			
Fusarium sp.			
Leptophaeria maculans	Blackleg	Yes	
Mycosphaerella brassicicola	Black ring spot	Yes	
Peronospora parasitica	Downy mildew	Yes	
Plasmodiophora brassicae	Club root	Yes	
Pseudocercosporella capsellae	White leaf spot	Yes	
Pseudomonas sp.			
Pseudomonas syringae pv. *maculicola*	Bacterial leaf spot		
Pyrenopeziza brassicae	Light leaf spot	Yes	
Rhizoctonia solani	Wire stem	Yes	
Sclerotinia sclerotiorum		Yes	
Turnip mosaic virus			
Turnip yellow mosaic virus			
Xanthomonas campestris pv. *raphani*	Bacterial leaf spot	Yes	
Xanthomonas campestris pv. *campestris*	Black rot	Yes	
Calendula officinalis (marigold)			
Alternaria alternate		Yes	
Alternaria porri			
Botrytis cinerea	Grey mould	Yes	
Curvularia pallescens			
Drechslera hawaiiensis			
Fusarium spp.			
Callistephus chinensis (Chinese aster)			
Alternaria alternate	Seedling blight	Yes	
Alternaria zinniae		Yes	
Ascochyta asteria	Leaf spot		
Botrytis cinerea	Grey mould	Yes	
Chrysanthemum mosaic virus			
Fusarium oxysporum f. sp. *callistephi*	Aster wilt	Yes	Armitage, 1993
Gibberella avenacea			
Phoma sp.	Seedling blight		
Rhizoctonia solani			
Septoria callistephi	Leaf spot		
Stemphylium callistephi	Leaf spot		
Celosia sp (cockscomb)			
Lilac ring mottle virus			
Spinach latent virus			Richardson, 1990
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	Bacterial wilt	Yes	Maude, 1996

Ornamental species and pathogen	Disease	Pathogen reported in the UK*	Reference**
<u>*Chrysanthemum* spp. (chrysanthemum)</u>			
(including *Dendranthema* spp. and florist's chrysanthemum)			
Alternaria chrysanthemi			
Alternaria sp.			
Alternaria zinniae		Yes	
Botrytis cinerea	Grey mould	Yes	
Chrysanthemum stunt viroid		Yes	
Didymella ligulicola	Ray blight	Yes	
Fusarium spp.			
Phoma sp.			
Rhizoctonia solani	Damping off	Yes	
Sclerotinia sclerotiorum		Yes	
<u>*Consolida* spp. (delphinium, larkspur)</u>			
Coniothyrium hellebori			
Diaporthe arctii	Stem canker		
Erwinia carotovora ssp. *atroseptica*		Yes	
Pseudomonas syringae pv. *delphinii*	Bacterial leaf spot	Yes	
<u>*Cyclamen persicum* (cyclamen)</u>			
Botrytis cinerea	Grey mould		O'Neill, 2006
Colletotrichum gloeosporioides	Anthracnose		O'Neill, 1987
Fusarium oxysporum f. sp. c*yclaminis*	Wilt		Tompkins & Snyder, 1972 Daughtrey *et al.,* 1995
Potato virus X			
Ramularia cyclaminicola	Leaf spot/stunt		
Septoria cyclaminis	Leaf spot		
Tomato mosaic virus			
<u>*Dahlia variables* (dahlia)</u>			
Gibberella avenacea			
Myrothecium roridum			
Sclerotinia sclerotiorum		Yes	
<u>*Dianthus* spp. (carnation, sweet william, pinks)</u>			
Alternaria dianthi	Leaf spot		
Alternaria dianthicola	Leaf spot	Yes	
Alternaria saponariae			
Botrytis cinerea	Grey mould	Yes	
Carnation cryptic virus			
Fusarium spp.			
Rhizoctonia solani		Yes	
<u>*Digitalis* spp. (foxglove)</u>			
Colletotrichum fuscum	Anthracnose		
Septoria digitalis			
<u>*Gerbera jamesonii* (African daisy, gerbera)</u>			
Alternaria porri			
Alternaria zinniae		Yes	
Botrytis cinerea	Grey mould	Yes	Orlikowski *et al.,* 1974

Ornamental species and pathogen	Disease	Pathogen reported in the UK*	Reference**
Helianthus annuus (sunflower)			
Alternaria alternate			
Alternaria carthami			
Alternaria helianthi			
Alternaria zinniae		Yes	
Botrytis cinerea	Grey mould	Yes	
Leptosphaeria linquistii			
Macrophomina phaseolina	Charcoal rot	Yes	
Plasmopora halstedii	Downy mildew		
Pseudomonas syringae pv. *helianthi*	Bacterial blight		Masselli *et al.,* 2002
Pseudomonas syringae pv*. tagetis*			
Puccinia helianthi	Rust		
Sclerotinia sclerotiorum		Yes	
Septoria helianthi			
Stromatinia subularis			
Sunflower rugose mosaic virus			
Tomato black ring virus			
Verticillium albo-atrum	Wilt	Yes	
Verticillium dahliae	Wilt	Yes	
Impatiens spp. (Busy Lizzie or snapweed)			
Alternaria zinniae		Yes	
Phyllosticta impatientis			
Plasmopora obducens	Downy mildew	Yes	Jones & O'Neill, 2005
Rhizoctonia solani			
Lavatera spp.			
Colletotrichum sp.	Anthracnose	Yes	Maude, 1994
Lobelia			
Alternaria alternate	Leaf spot and	Yes	Baker 1972 (p.28)
	stem rot	Yes	Hall & Taylor, 1983
Lupinus spp. (lupin)			
Bean yellow mosaic virus		Yes	
Botrytis cinerea	Grey mould	Yes	
Colletotrichum acutatum	Anthracnose	Yes	Reed *et al.*, 1996
Cucumber mosaic virus			
Diaporthe woodii	Phomopsis stem blight		
Erwinia sp.			
Fusarium oxysporum	Wilt		
Gibberella avenacea			
Glomerella cingulata	Anthracnose	Yes	
Pea mosaic virus			
Peanut mottle virus			
Peanut stunt virus			
Pleiochaeta setosa	Brown spot	Yes	
Pseudomonas spp.			
Sclerotinia sclerotiorum		Yes	
Stemphylium sp	Leaf spot		
Verticillium albo-atrum	Wilt	Yes	
Verticillium sp.	Wilt		

Ornamental species and pathogen	Disease	Pathogen reported in the UK*	Reference**
Matthiola (garden stock)			
Alternaria raphani	Leaf spot		
Alternaria spp.	Leaf spot		
Botrytis cinerea	Grey mould	Yes	
Fusarium oxysporum f. sp. *mathioli*	Wilt	Yes	
Gibberella avenacea		Yes	
Phoma matthiolicola			
Xanthomonas campestris	Black rot	Yes	
Xanthomonas campestris pv. *incanae*	Bacterial blight		
Meconopsis (Chinese poppy)			
Peronospora arborescens	Downy mildew	Yes	
Nicotiana spp. (tobacco)			
Alternaria alternate			
Alternaria longipes			
Alternaria zinniae		Yes	
Arabis mosaic virus			
Arracacha virus A			
Artichoke yellow ringspot virus			
Asparagus virus 2			
Botrytis cinerea	Grey mould	Yes	
Cassava green mottle virus			
Cercospora nicotianae	Green spot		
Cherry leaf roll virus			
Colletotrichum tabacum	Anthracnose		
Corynebacterium fascians			
Eggplant mosaic virus			
Erwinia carotovora spp. *carotovora*	Tobacco hollow stalk		
Erwinia spp.			
Nicotiana velutina mosaic virus			
Pelargonium zonate spot virus			
Peronospora tabacina	Downy mildew	Yes	
Potato (Andean) latent virus			
Potato virus U			
Pseudomonas aeruginosa	Phillipine leaf spot		
Pseudomonas syringae pv. *mellea*	Wisconsin leaf spot		
Pseudomonas syringae pv. *tabaci*	Wildfire		
Rubus Chinese seed-borne virus			
Spinach latent virus			
Tobacco ascending necrosis virus			
Tobacco etch virus			
Tobacco mosaic virus			
Tobacco ringspot virus			
Tobacco streak virus			
Tobacco black ring virus			
Tomato ringspot virus			
Xanthomonas heterocea	Leaf spot		

Ornamental species and pathogen	Disease	Pathogen reported in the UK*	Reference**
Pelargonium sp. (pelargonium, geranium)			
Pseudomonas sp.	Leaf spot	Yes	Englehard *et al.,* 1983
Tobacco ringspot virus			
Tomato ringspot virus			
Petunia spp. (petunia)			
Arabis mosaic virus			
Artichoke yellow ringspot virus			
Asparagus virus 2			
Eggplant mosaic virus			
Raspberry ringspot virus			
Tomato black ring virus			
Tobacco mosaic virus			
Tobacco ringspot virus			
Phlox drummondii (phlox)			
Alternaria sp.			
Cochliobolus lunatus			
Rhizoctonia solani	Damping off		
Septoria drummondii	Leaf spot	Yes	Wenham, 1958
Primula spp. (primrose, polyanthus)			
Botrytis cinerea	Grey mould	Yes	Barnes & Shaw, 2003
Phyllosticta primulicola	Leaf spot		
Pseudomonas syringae pv. *primulicola*	Bacterial leaf spot		
Senecio cruentus (cineraria)			
Alternaria cinerariae	Leaf spot	Yes	
Botrytis cinerea	Grey mould	Yes	
Erysiphe cichoracearum	Powdery mildew	Yes	
Cineraria mosaic virus			
Solanum spp. (excluding *S. dulcamara, S melongena, S tuberosum)*			
Potato spindle tuber viroid			
Strawberry latent ringspot virus			
Tagetes (marigold)			
Alternaria tagetica			
Alternaria zinniae	Leaf blight	Yes	
Fusarium oxysporum f. sp. *callistephi*	Wilt		
Rhizoctonia solani	Damping off	Yes	
Septoria tageticola	Leaf spot		
Pseudomonas syringae pv. tagetis	Bacterial leaf spot		
Verbena (verbena)			
Alternaria alternate			
Phoma exigua			
Viola (pansy, violet)			
Cherry leaf roll virus			
Colletotrichum violae-tricoloris	Anthracnose		

Ornamental species and pathogen	Disease	Pathogen reported in the UK*	Reference**
Mycocentrospora acerina	Halo blight	Yes	
Phoma sp.			
Ramularia lacteal	Leaf spot	Yes	
Rhizoctonia solani		Yes	
Sphaceloma violae	Scab		
Tobacco rattle virus			
Urocystis violae	Smut		
Zinnia elegans (zinnia)			
Alternaria zinniae	Leaf spot/blight	Yes	Beaumont *et al.*, 1958 Franklin & Goodwin, 1982
Asparagus virus 2			
Botrytis cinerea	Grey mould	Yes	
Colletotrichum acutatum			Kulshrestha, 1976
Erysiphe cichoracearum	Powdery mildew		
Glomerella cingulata			
Phyllosticta sp.			
Rhizoctonia solani	Damping off		
Tobacco ringspot virus			
Tomato black ring virus			
Xanthomonas campestris pv. *zinniae*			

*****Pathogen recorded in the UK but not necessarily on the listed host.
**Richardson (1990) unless stated otherwise.

Table 3: List of diseases of common bedding and pot plant species grown in the UK that are suspected to be seed-borne

Ornamental species and pathogen	Disease	Source
Alyssum		
Pseudomonas sp.	Leaf spot	M. McPherson, pers. comm.
Lisianthus		
Peronospora chlorae	Downy mildew	T. Brokenshire, pers. comm.
Fusarium oxysporum	Wilt	T O'Neill, unpublished
Primula spp.		
Ramularia agrestis	Leaf spot	M. McPherson, pers. comm.
Salvia spp.		
Pseudomonas spp.	Leaf spot	M. McPherson, pers. comm.
Viola		
Ramularia agrestis	Leaf spot	M. McPherson, pers. comm.

Table 4: Summary of some key features of selected seed-borne diseases of protected bedding and pot plant species

Crop and disease	Recent occurrence of the disease	Potential damage	Favourable conditions for disease development
Alyssum			
Alternaria leaf spot	Uncommon	Moderate	-
Sclerotinia stem rot	Quite common	Severe	-
Stemphylium leaf spot	Uncommon	Mild	-
Bacterial leaf spot*	Quite common	Mild-severe	Warm and wet
Antirrhinum			
Phyllosticta leaf spot	Rare	Severe	
Pseudomonas leaf spot	Occasional	Moderate	Wet leaves; heavy rain
Rust	Quite common	Severe	Wet leaves
Cheiranthus			
Xanthomonas blight	Quite common	Severe	Wet leaves; splash-borne
Cyclamen			
Anthracnose	Uncommon	Severe	Warm and humid
Grey mould	Common	Mild-severe	Cool and moist
Fusarium wilt	Quite common	Severe	Very warm
Impatiens			
Downy mildew	Sporadic	Very Severe	Cool and moist
Lobelia			
Alternaria leaf spot and stem rot	Common	Mild to severe	Cool and moist
Lupin			
Anthracnose	Common	Very severe	Warm; wet leaves, water-splash
Nicotiana			
Downy mildew	Quite common	Severe	Cool and humid
Pelargonium			
Grey mould	Common	Severe	Cool and humid
Pseudomonas leaf spot	Uncommon	Moderate	-
Phlox			
Septoria leaf spot	Quite common	Severe	Overhead watering
Primula			
Grey mould	Common	Severe	Cool and moist
Ramularia leaf spot*	Common	Mild-severe	Cool and moist
Senecio			

Crop and disease	Recent occurrence of the disease	Potential damage	Favourable conditions for disease development
Alternaria leaf spot	Quite common	Moderate	Warm and wet leaves; water splash
<u>Viola</u>			
Mycocentrospora leaf spot	Uncommon		
Ramularia leaf spot*	Common	Moderate	Wet leaves
<u>Zinnia</u>			
Alternaria blight	Common?	Moderate/severe?	
Botrytis stem rot	Common	Severe	Cool and humid

*Suspected but not confirmed as seed-borne

Review of selected seed-borne diseases

It should be noted that the majority of the chemical seed treatments described in this section are experimental uses and not approved uses. Seed treatments will be reviewed in more detail in Year 2 of the project.

Alyssum

<u>Alternaria leaf spot (*Alternaria* sp.)</u>

This fungus was identified on seed of alyssum in 1997 and caused damage to cotyledon leaves and hypocotyls (Maude/Coutts pers. comm.). It was recovered from 0.5-2% of 200 seeds after surface sterilisation, indicating it was probably located in the testae of seeds.

<u>Sclerotinia stem rot (*S. sclerotiorium*)</u>

This fungus was found contaminating 5% of seed of cv. Snow Crystals tested in 1994 (Coutts/White pers. comm.) and one seed of one small sample tested in 1997 (Maude/Coutts, pers. comm.). There are occasional Plant Clinic reports of such infection occurring (McPherson, pers. comm.).

<u>Leaf spot (*Stemphylium botryosum*)</u>

Stemphylium botryosum, (the asexual stage of *Pleospora herbarum*) was recovered from 2-2.5% of seed tested after surface sterilisation in 1997 (Maude/Coutts, pers. comm.) It is generally considered a weak pathogen, although it may cause damping-off of seedlings. It is possible that there is some host specialisation in this fungus and further work is required on this aspect.

Bacterial leaf spot (*Pseudomonas* sp.)

A bacterial leaf spot is occasionally seen in alyssum and is associated with infection by a *Pseudomonas* sp. It is considered that it may be seed-borne although this has not been validated (McPherson, pers. comm.). The association of bacteria with yellowing and subsequent collapse of cv. Snowdrift is being investigated.

Antirrhinum

Leaf spot and stem rot (*Phyllosticta antirrhini*)

The seed-borne nature of this disease was first demonstrated in the USA and subsequently in the UK (Rosser, 1961; Simpson *et al.*, 1971; Baker, 1972). Pycnidia of *P. antirrhini* were found on seed coats and on brown lesions on the hypocotyls and cotyledons of seedlings (Rosser, 1961). The disease was troublesome in the 1960s causing severe and extensive damping-off, leaf spots and malformation and distortion of older seedlings. The disease has rarely been seen in recent years.

Leaf spot and seedling blight (*Pseudomonas syringae* pv. *antirrhini*)

A bacterial leaf spot of antirrhinum caused by *Ps. syringae* pv. *antirrhini* (syn. *Ps. antirrhini*) was first noted in southern England in 1965 (Baker 1972), occurring after very heavy rain. Symptoms are initially small (*c*. 1 mm diameter) light brown spots with a narrow green margin. Fully developed spots are discrete, circular, 4-5 mm in diameter, sunken and papery brown with a well-defined dark brown margin, sometimes with a water-soaked zone around the lesion. The disease may kill whole leaves and spread down petals into the stem (Simpson *et al.*, 1971). Disease spread is rapid if seedlings are exposed to rain splash. Seed-borne infection was confirmed on a range of varieties. The disease has also been confirmed on other members of the *Scrophulariaceae*, notably calceolaria and penstemon (Moffett 1966). Antirrhinum varieties differ greatly in susceptibility. Dry heat for 8 h at 49°C was sufficient to destroy *Pseudomonas* on antirrhinum seed but it also tended to reduce seed germination (Simpson *et al.,* 1971).

Rust (*Puccinia antirrhini*)

Infection of 4-5% of seeds was reported in the USA.

Cheiranthus

Bacterial wilt (*Xanthomonas campestris*)

This disease was first confirmed on wallflowers in England in 1979. Affected plants are stunted, chlorotic, wilted and readily shed their basal leaves. Leaves may show a characteristic v-shaped black rot lesion. Badly affected plants may collapse and die. The stem vascular tissue shows a characteristic discolouration varying from light brown to almost black. Systemic infection has been noted in some plants with no obvious symptoms. Carry over on debris is considered to be the main source of inoculum of this disease. However, the bacterium was found on one (cv. Primrose Monarch) out of 16 seed lots tested in the UK in 1976 (Griffin & Baker, 1976). Transmission and control of *X. campestris* in seeds of *Matthiola* spp. and *Brassica* spp. is well documented (Kendrick & Baker, 1942; Wilson, 1942; Cook *et al*., 1952).

Cyclamen

Anthracnose (*Colletotrichum gloeosporioides*)

Symptoms of this disease include small brown leaf spots and stunted and distorted growth. Young leaves may develop blackened edges and fail to expand while older leaves show down-curling of leaf margins; flower buds turn dark brown or black and cease to expand and flowers collapse at the neck. In the later stages of development, orange-coloured spore masses ooze from petiole, peduncle and leaf lesions (O'Neill, 1987). *Glomerella cingulata* (asexual stage: *Colletotrichum gloeosporioides*) was isolated from 2% of cyclamen seed cv. Aida (O'Neill, unpublished).

Warm conditions and overhead watering favour the disease. The fungus is spread by water splash and wet leaves allow infection. *G. cingulata* also causes anthracnose on other woody and herbaceous ornamentals although information on the specificity of isolates from different hosts is lacking.

Grey mould (*Botrytis cinerea*)

Examination of cyclamen seed cvs Midori White and Midori Rose flame by a very sensitive molecular test specific for DNA of *B. cinerea* indicated infection levels of 76 and 33% respectively (O'Neill, 2006). Seeds were tested individually after crushing. When the same seed lots were examined by plating onto a botrytis-selective agar medium, no *B. cinerea* was recovered.

Fusarium wilt (*Fusarium oxysporum* f. sp. *cyclaminis*)

Symptoms include progressive yellowing and wilting of leaves until the whole plant collapses. Reddish brown discolouration of the vascular system occurs within the corms. Root rot may also occur. The seed-borne nature of this disease has not been conclusively demonstrated. Observations suggest it is introduced into a greenhouse on young plants and persists as a saprophyte. The fungus has been isolated from organic debris in seed packets (Daughtrey *et. al.,* 1995). Fusarium wilt remains a serious, sporadic disease in UK crops.

Impatiens

Downy mildew (*Plasmopara obducens*)

This disease results in pale green leaves with a white downy growth developing on the lower surface; lower leaves may become completely covered with the fungus. Premature leaf fall, stunted growth and plant collapse can result (Jones & O'Neill, 2005). The disease was first recorded in the UK in 2003 (Lane *et al.,* 2005) but seed-borne transmission in *Impatiens balsamina* in India had been previously reported (Sohi & Tyagi, 1974). When sown, infected seed gave rise to systemically infected plants. This disease, which caused widespread damage in commercial plantings in the UK in 2003, (e.g. civic floral displays), has occurred occasionally in subsequent years, including on seed-raised plants in 2007. It is suspected that the disease was initially brought into the country on vegetatively propagated material from overseas.

Lavatera

Anthracnose (*Colletotrichum* sp.)

A *Colletotrichum* species, identified as belonging to the *C. orbiculare* group, was isolated from three out of eight commercial lots of lavatera seed (Maude, 1994). Levels of infection were 3.3% (cv. Silver Cup), 6% (cv. Mont Blanc) and 18.7% (cv. Mont Blanc). Dissection followed by plating of seed parts onto agar showed that, for the worst-affected lot, around 20% of seeds bore the fungus within the seed coat and endosperm while 3% had affected embryos. This showed that for effective control by fungicide seed treatment, it would be necessary for the treatment to penetrate seed tissues. Preliminary laboratory tests of fungicides on naturally infected lavatera seed indicated that prochloraz applied alone or combined with carbendazim, and thiram combined with benomyl or thiabendazole, eradicated seed-borne infection without harming the germination of seeds. The fungicides were applied at 1 g a.i./kg of seeds. Propiconazole at this rate greatly reduced seedborne infection but also significantly reduced germination.

Lobelia

Alternaria leaf spot and stem rot (*Alternaria alternata*)

Alternaria alternata can cause severe seedling blight, damping off, leaf spotting and stem rotting of lobelia. Disease transmission has been demonstrated from naturally infected seed (Wilcox, 1963). The level of disease varies greatly from year to year and with variety. Some trailing varieties have been badly affected e.g. White Fountains. Good control has been achieved with an aerated steam treatment at 50-51ºC for 15-20 minutes; a thiram soak was ineffective and reduced germination (Hall & Taylor, 1983). Iprodione is also used to control seed-borne infection, although some isolates of the fungus are resistant to this fungicide (O'Neill & Griffin, 1991).

Lupin

Anthracnose (*Colletotrichum* spp.)

Anthracnose of ornamental lupin is attributed to *Colletotrichum acutatum* (Reed *et al.,* 1996), while on field lupins the disease has been attributed to both *C. acutatum* and *Colletotrichum gloeosporioides* (teleomorph: *Glomerella cingulata*). More recently, the name *Colletotrichum lupini* was proposed for the pathogen causing lupin anthracnose based on DNA sequencing (Nurenberg *et al.,* 2002). The fungus causes necrotic lesions on leaf blades, petioles, stems and flower stalks and results in distorted growth. Under moist conditions a mass of orange coloured spores develop on lesions. Seed-borne transmission has been reported for both fungi. Reed *et al.* (1996), reported infection by *C. acutatum* on three out of 14 ornamental lupin seed samples tested (cvs Gallery Red Shades, Gallery Mixed and Mirakel) at levels of 0.3-1.6%. For control of *C. lupini* on field lupin, application of dry heat for 4-7 days at 15ºC or up to 4 days at 70ºC will significantly reduce and possibly eliminate anthracnose infection in lupin seed; temperatures below 70ºC had little or no effect on germination of *L. angustifolius* (Thomas & Adcock, 2004).

Nicotiana

Downy mildew (*Peronospora tabacina)*

This disease can be systemic and cause considerable damage. Evidence of seed-borne infection has been reported in Australia and Russia; oospores were found in the testas of cv. Samsun in Russia. Thiram seed treatment may give some control.

Pelargonium

Grey mould (*Botrytis cinerea*)

B. cinerea causes a range of symptoms including spots and rot of leaf and petal tissues, a stem base rot of cuttings and damping off of seedlings. Lesions caused by *B. cinerea* are recognised by the characteristic grey, furry sporulation. Leaf lesions often develop a zonate pattern and are often initiated by flower petals dropping on them.

Bacterial leaf spot (*Pseudomonas* sp.)

Koucheki (1973a,b), reported seed-borne transmission of a *Pseudomonas* sp. on *Pelargonium* x *hortorum* in the USA. A leaf spot of pelargonium caused by *Pseudomonas cichorii* was described by Engelhard *et al.* (1983), but no information was presented on the role of seed in the disease epidemiology. When exposed to occasional wetting, leaf lesions were 2-10 mm in diameter with a tan centre, a dark margin and a chlorotic halo. Frequently infection only occurred on the leaf margin. When exposed to rain, the disease was characterised by dark brown to black irregular, water soaked spots around 5 mm in diameter, developing to larger, necrotic areas. With extensive infection, the entire leaf became necrotic. Infected flower buds turned black and fail to open. No stem symptoms have been observed. An isolate of *Ps. cichorii* from pelargonium was pathogenic to cabbage, cauliflower and chrysanthemum. In the UK, isolates of *Pseudomonas marginalis* have been obtained from leaf spots on seed-raised geranium; the role of seeds in disease transmission is uncertain.

Phlox

Septoria leaf spot (*Septoria drummondii*)

This disease causes diffuse chlorotic blotches, turning light brown, on one or both cotyledons and subsequently lesions on true leaves. Damage may also occur on stems, petioles, peduncles and sepals. Small, dark coloured spore cases are visible within the leaf spots. Experiments in New Zealand demonstrated the seed-borne nature of the disease, with both superficial and deep-seated infection (Wenham, 1958)

Primula

Grey mould (*Botrytis cinerea*)

This common disease causes leaf and flower rotting. Affected tissues develop a grey furry mould under humid conditions. Infection is often found on lower leaves in contact with the growing medium, and on leaves where flower parts have fallen. Infection of commercial hybrid primula seed with *Botrytis cinerea* (at levels up to 33% in non-sterilised seed) was

recently demonstrated (Barnes & Shaw, 2003). Symptomless systemic infection was noted in roots, leaves and flowers.

Senecio cruentus (*Cineraria*)

Alternaria leaf spot (*Alternaria cinerariae*)

Leaf spots are initially small, dark and water-soaked, turning a reddish or olive brown colour as they enlarge to around 1 cm. The centres of spots may turn grey, and dark lesions develop on petioles. Leaf spots sometimes coalesce to kill leaves. The fungus only affects cineraria. The disease is encouraged by extended periods of leaf wetness (Cooper, 1956; Daughtrey *et al.*, 1995).

Viola

Mycocentrospora leaf spot (*Mycocentrospora acerina*)

Seed-borne transmission was demonstrated by Gill (1971).

Ramularia leaf spot (*Ramularia lactea*)

Seed-borne infection by *Ramularia lactea* is believed to occur (Pirone, 1960).

Zinnia

Alternaria blight (*Alternaria zinniae*)

Alternaria zinniae occurs frequently in seed and sometimes destroys leaves and flowers. Of 25 varieties tested in England in 1956, none had seed that was entirely free of infection, with the level of infection ranging from 20 to 85% (Beaumont *et al.,* 1958). The fungus is present in all parts of the seed; deep-seated infection causes pre-emergence death while superficial infection causes seed disease after emergence. Recommended seed treatments include mancozeb, thiram, heat therapy with steam/air (30 mins at 60ºC) and hot water treatment (30 mins at 52ºC followed by cold water). Thiram dust treatment gave excellent control without reducing seed germination (Beaumont *et al.,* 1958).

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

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

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.

Visual examination of dry seed

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

Examination of seed by agar plate method

<u>Growth media</u>

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 5.

Table 5: 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.

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.

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.

A pure culture of an unknown sclerotial forming fungus that developed consistently on lobelia seed was also sent to CSL for identification.

Pathogenicity tests

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

Alternaria species from wallflower seed

Ten wallflower plants were potted on into 9 cm pots containing M2 compost. All plants were placed in gravel trays in a glasshouse (set at 20°C) and watered to the base.

A spore suspension (1.3 x 10^5 spores per mL) was prepared from a 28-day-old pure culture of *Alternaria cheiranthi* (isolated from wallflower seed) grown on PDA at 20-22°C. Five plants were placed into individual plastic bags and inoculated with approximately 5 ml of the spore suspension using a hand mister. Five plants were placed into individual plastic bags and inoculated with water as a control. All plastic bags were sealed and the sealed plants were placed into a controlled environmental cabinet (set at 20°C, 12 h light / 12 h dark) for 3 days. After this time, all plants were returned to the glasshouse where the bags were removed and the pots were placed into gravel trays.

All plants were assessed for symptom development after a further 5 days (a total of 8 days after inoculation).

Unidentified bacterial species

From ADAS seed tests, eight bacterial isolates from non-sterilised pelargonium seed were cultured on PDA. The eight isolates as pure cultures were used to each inoculate five plants of zonal geranium. For each isolate, a sterile needle was streaked in the bacterial culture then used to stab inoculate the stem base and one leaf of each of five plants. Five uninoculated control plants were set up by stab wounding with a sterile needle dipped in sterile distilled water. Inoculation sites were marked to allow subsequent monitoring. The plants were individually placed in sealed polythene bags to maintain 100% RH for 48 h, then removed from bags and transferred to a glasshouse (20°C). The plants were assessed after 1 week and were subsequently monitored for approximately 4 weeks.

From STC seed tests, twelve bacterial isolates were collected from four seed batches as detailed in Table 6.

Table 6. Unidentified bacterial isolates collected following STC seed tests

Crop	Bacterial isolates collected	Collected from sterilised (S) or unsterilised (US) seed	Description
Antirrhinum	W4	US	White colonies
	C-Y2	US	Creamy-yellow
	C1	US	Cream
	AY3	US	Yellow
Geranium	C2	S	Cream
	W1A	S	White A
	W1B	S	White B
	GY	US	Yellow
Wallflower	C6	US	Cream
	W1	US	White
	Y	US	Yellow
	W5A	US	Yellow-orange
Salvia	W2A	US	White
	SC6	US	Cream
	Y3	S	Yellow
	SW1	S	White

Pathogenicity tests using each isolate on seedlings of the appropriate host were set up. The isolates were tested on five damaged and five undamaged seedlings, alongside uninoculated, damaged and undamaged control plants. Bacterial isolates were grown on NDA at 23°C for 2-3 days. The seedlings were damaged using sterile needles and inoculated with 10µl loop of each bacteria in solution with SDW.

Streptobotrys streptothrix from lobelia seed

A pathogenicity test is ongoing. Methods and results will be reported in the year 2 Annual Report.

Prevalence of seed-borne pathogens in 2006

Recovery of fungi and bacteria from seed

Twenty-nine commercial seed lots from 18 ornamental species were tested for seed-borne fungal and bacterial pathogens. Seeds were not tested for virus infection. The incidences of pathogen recovery for each seed lot are listed in Tables 7 (ADAS tests) and 8 (STC tests). It should be noted that the use of a specific agar medium to aid recovery of a particular target pathogen may result in failure to detect another pathogen that may be present on the seed. For all seed lots except four, surface sterilisation resulted in an increase in the incidence of 'clean seed', largely due to reduced contamination from saprophytic fungi and non-pathogenic bacteria.

Of the potential seed-borne pathogens being tested for on seed from different ornamental species, *Botrytis cinerea, Alternaria* species and *Pythium* sp. were the only fungal pathogens recovered.

B. cinerea was listed as a potential seed-borne pathogen for only two of the crops tested (primula and zinnia) but was actually isolated from ten seed lots of the following crops: antirrhinum, pelargonium, lobelia, lupin, pansy, phlox, wallflower, stock, impatiens and zinnia. In the case of pelargonium, lupin, pansy, phlox and zinnia, *B. cinerea* was isolated from surface sterilised seed, suggesting more deep-seated seed infection. 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, but were not detected on lobelia or lavender. There was a high incidence of an *Alternaria* species (up to 40% infection) on each of two seed lots of zinnia, from both surface sterilised and non-surface sterilised seed. The pathogenicity of this *Alternaria* species on zinnia has not yet been confirmed. On one seed lot of cineraria, the target pathogen, *A. cinerariae,* was recovered at a low incidence (1%). A high incidence of *A. cheiranthi* was recovered from surface sterilised and non-surface sterilised seed of wallflower (one seed lot). Pathogenicity of this fungus on wallflower was subsequently confirmed (Section 2.5.2). An unidentified *Alternaria* species was recovered from one lot of aquilegia seed.

Pythium was listed as a potential seed-borne pathogen for stock and aquilegia. A *Pythium* species was isolated at a low incidence (0.3%) only from one lot of aquilegia seed.

A fungus isolated from 6 out of 300 non-surface sterilised lobelia seed (seed batch 5, Table 7) was identified by a molecular test (ITS sequencing) as *Streptobotrys streptothrix* (C. Lane, pers. comm.). A characteristic feature of the fungus was development of abundant small black sclerotia, less than 2 mm in diameter (Figure 2.1). Information available on both the sclerotial stage and the anamorph (*Botrytis streptothrix*) is scant. The species has not been previously recorded in the UK but has been reported from North Eastern USA on *Arisaema* spp., *Hydrastic*, *Oronitium* and *Symplocarpus* (C. Lane, pers. comm.). A pathogenicity test using this fungus is ongoing.

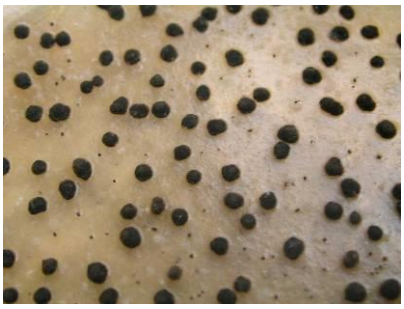

Figure 2.1. Sclerotia of *Streptobotrys streptothrix* isolated from lobelia seed

For seed batches of crops where bacterial species were the target pathogen, representative cultures of bacteria isolated were sent to CSL for identification. From ADAS seed tests, bacteria occurring on seed from antirrhinum (one isolate), wallflower (two isolates) and geranium (two isolates), from seed batches 1, 10 and 14 respectively in Table 7 were identified by CSL. None of the isolates were identified as the target pathogens *Xanthomonas* and/or *Pseudomonas* species. The Fatty Acid Profile Report (Appendix 1) provides an indication of species present. From STC seed tests, three bacterial isolates from geranium seed were identified by Fatty Acid Profiling as *Bacillus* species (see Section 2.5.2).

Pathogenicity tests

For wallflower, all five plants inoculated with *Alternaria cheiranthi* developed lesions on the leaves (Figure 2.2) and subsequently leaf yellowing whilst the control plants remained unaffected. A selection of representative leaf lesions were plated out onto PDA+S and incubated for 9 days at 20ºC. *A. cheiranthi* was identified on 3 out of 10 leaf pieces. This study confirms that *A. cheiranthi* pathogenic to wallflower can be seed-borne.

Figure 2.2. Symptoms of black mould on wallflower following inoculation with an isolate of *Alternaria cheiranthi* from wallflower seed

Geranium plants inoculated with each of eight bacterial isolates from pelargonium (ADAS seed tests) remained symptomless, indicating that these unidentified species were non-pathogenic.

From STC pathogenicity studies using bacterial isolates, the majority of tests did not result in any symptoms to suggest that the isolates under investigation were pathogenic. Some leaf distortion and death of the inoculated leaf was seen in the geranium seedlings which were damaged and inoculated with isolates GY, W1A and W1B (Figure 2.3). Fatty acid profiling suggested that these isolates were all *Bacillus* species (possibly *B. megaterium*, *cereus* or *pumilis*). These species do not usually behave as plant pathogens.

Figure 2.3. Distortion and death of geranium leaves inoculated with bacterial isolate code: GY (*Bacillus* sp.) from geranium seed

Table 7: Recovery of fungi and bacteria from ornamental seeds - ADAS tests, October 2006

	Crop	Target plant pathogen(s)	Incidence of seeds (of 300) from which suspect plant pathogens recovered		Number of 'clean' seeds (of 300)	
			Surface sterilised	Not surface sterilised	Surface sterilised	Not surface sterilised
1.	Antirrhinum	*Pseudomonas syringae*	0	0	268	18
2.	Cineraria	*Alternaria cinerariae*	0	4 *Alternaria cinerariae*	0	0
3.	Cyclamen	*Fusarium oxysporum*	0*	0	298	286
4.	Pelargonium	*Pseudomonas* and *Xanthomonas spp.*	1 unidentified bacteria*** 1 *B. cinerea*	8 unidentified bacteria*** 1 *B. cinerea*	327	311
5.	Lobelia	*Alternaria alternata* and *Sclerotinia sclerotiorum*	0	6 sclerotial fungus 7 *B. cinerea*	252	30
6.	Lupin	*Colletotrichum acutatum*	0** 1 *B. cinerea*	0	101	137
7.	Pansy	*Ramularia lactis* and *R. agrestis*	0 20 *B. cinerea*	0 9 *B. cinerea*	23	0
8.	Phlox	*Septoria drummondii*	0 4 *B. cinerea*	0	206	0
9.	Primula	*Botrytis cinerea*	0	0	254	0
10.	Wallflower	*Xanthomonas campestris* pv. *Campestris*	0	0 1 *B. cinerea*	251	174
11.	Aquilegia	*Alternaria / Pythium*	2 *Alternaria* & 1 *Pythium*	15 *Alternaria* only	236	61
12.	Lavender	*Alternaria / Rhizoctonia*	0	0	272	196
13.	Stock	*Fusarium / Pythium*	0 2 *Aspergillus sp.*	0 1 *B. cinerea*	137	0
14.	Geranium	*Pseudomonas* and *Xanthomonas spp.*	0	0 1 *Aspergillus sp.*	255	75
15.	Impatiens	*Pythium*	0	0 2 *B. cinerea*	150	0
16.	Zinnia	*Alternaria* and *Botrytis cinerea*	106 *Alternaria* and 3 *B. cinerea*	115 *Alternaria* and 6 *B. cinerea*	18	0
17.	Wallflower	*Alternaria cheiranthi*	33 Alternaria	92 Alternaria	234	0

Notes:
'Clean seed' - no pathogens or saprophytes recovered.
* 298 seed tested; ** 135 seed tested ; *** 330 seed tested
Use of selective agar media for target plant pathogens may result in failure to detect some non-target plant pathogens.

Table 8: Recovery of fungi and bacteria from ornamental seeds - STC tests, February 2007

	Crop	Target plant pathogen(s)	Incidence of seeds (of 300) from which suspect plant pathogens recover		Number of 'clean' seeds (of 300)	
			Surface sterilised	Not surface sterilised	Surface sterilised	Not surface sterilised
1.	Antirrhinum	*Pseudomonas syringae*	0	Possible bacterial pathogens* *1 B. cinerea*	299	268
2.	Cineraria	*Alternaria cinerariae*	0	0	297	238
3.	Cyclamen	*Fusarium oxysporum*	0	0	298	288
4.	Pelargonium	*Pseudomonas* and *Xanthomonas* spp.	Possible bacterial pathogens*	Possible bacterial pathogens*	297	297
5.	Lobelia	*Alternaria alternata* and *Sclerotinia sclerotiorum*	0	0	300	293
6.	Lupin	*Colletotrichum acutatum*	2	0	278	287
7.	Pansy	*Ramularia lactis* and *R.agrestis*	0	0	281	61
8.	Phlox	*Septoria drumondii*	0	0	280	118
9.	Primula	*Botrytis cinerea*	0	0	32	0
10.	Wallflower	*Xanthomonas campestris*	Possible bacterial pathogens*	Possible bacterial pathogens*	295	198
11.	Salvia	*Pseudomonas* sp.	Possible bacterial pathogens*	Possible bacterial pathogens*	214	0
12.	Zinnia	*Alternaria* sp. and *Botrytis cinerea*	45 Alternaria	120 Alternaria	65	0

Notes:
'Clean seed' – no pathogens or saprophytes recovered.
* Bacterial isolates are being tested for their pathogenicity to the seedlings; results will be reported in year 2.
Use of selective agar media for target plant pathogens may result in failure to detect some non-target plant pathogens.

Conclusions

- Lists of confirmed and suspected seed-borne diseases of common bedding and pot plant species grown in the UK, have been compiled.
- A total of 246 pathogens affecting 36 hosts are listed as seed-borne.
- Standard methods to test for key seed-borne pathogens of important UK ornamental crops were determined.
- Twenty-nine commercial seed lots from 18 ornamental species were tested for seed-borne pathogens in project 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 the potential pathogens on seed from different ornamental species, *Botrytis cinerea, Alternaria* species, *Colletotrichum acutatum* and a *Pythium* species were the only 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); pathogenicity of the recovered *Alternaria* species to zinnia is to be tested. A pathogenicity test confirmed that *Alternaria cheiranthi* pathogenic to wallflower can survive on wallflower seed.
- Pathogenic bacterial species (*Pseudomonas* and *Xanthomonas* species) have not as yet been 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. Pathogenicity to lobelia is being tested.

Technology transfer

- Project meeting, STC, 15 February 2006.
- Project meeting, STC, 6 February 2007.

Acknowledgements

We are grateful to Coletta & Tyson Ltd and WJ Findon & Son Ltd for donation of seed samples.

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APPENDICES

Letter sent to seed companies, May 2006.

12 May 2006

Dear

New HDC Project: Seed-borne diseases of protected ornamentals – detection, prevalence and control (PC 252)

I am writing to draw your attention to a new HDC-funded project on seed-borne diseases of ornamentals and to invite your support by supply of information and / or seed samples.

The project has arisen because of:

1. the lack of a single, accessible reference source providing up-to-date information on pathogens either known or suspected or being seed-borne;
2. on-going sporadic outbreaks of diseases on UK nurseries, sometimes causing substantial losses, where circumstantial evidence suggests a seed-borne origin (e.g. appearance of disease at or soon after emergence);
3. increased production of seed outside of Europe and the consequent possibility of introducing new seed-borne pathogens new to the UK;
4. the lack of any significant (non-commercial) research, development or technology transfer work on this topic for many years (apart from ad hoc consultancy testing by ADAS, STC and some other laboratories).

The work will be undertaken jointly by ADAS and STC with myself as overall Project Leader, Stuart Coutts as Project Consultant and Fay Richardson as Project Coordinator. Coletta & Tyson and W J Findon & Son are industry partners.

We shall investigate culturable fungal and bacterial pathogens affecting bedding and pot plant species important to UK growers. This will include *Alternaria, Botrytis, Colletotrichum* etc, but not downy mildew or other non-culturable pathogens. Downy mildew is being investigated, on impatiens, in a separate HDC project (PC 230) by CSL and STC.

Broadly, the project aims are:

<u>Year 1 (April 2006 to March 2007)</u>

1. List known seed-borne diseases of key bedding and pot plant species.

2. Determine prevalence of seed-borne fungal and bacterial pathogens on up to 30 lots of commercial seed.

Year 2 (April 2007 – March 2008)

1. Determine prevalence of seed-borne fungal and bacterial pathogens on up to 30 lots of commercial seed.
2. Review the literature and identify promising chemical and non-chemical methods for control of seed-borne pathogens (e.g. by reference to work on vegetable seeds).
3. Test potential seed treatment on some seed-borne pathogens using naturally infected seed where possible.
4. Test the pathogenicity of selected fungi/bacteria isolated from seed.

Year 3 (April 2008 – March 2009)

1. Determine prevalence of seed-borne fungal and bacterial pathogens on up to 30 lots of commercial seed.
2. Further tests on seed treatments.
3. Write a Factsheet for growers on seed-borne diseases of ornamentals.

We envisage you may be able to help by:

a) Supply of naturally-infested seed for use in seed-treatment experiments in years 2 and 3;

b) Supply of information concerning seed treatment you currently use and promising, commercially useful new treatments.

A wide range of seed lots supplied by growers will be used for routine testing (years 1-3). Any additional seed supplied by participating seed companies (years 2-3) will be used primarily for experiments on control. In all cases, information relating to results on individual seed lots will be kept confidential with respect to seed source. Seed companies will each be given a unique reference code (known only to the seed company, the lead researcher and HDC); variety names will also be coded (e.g. Alyssum ZX 235).

Help provide to the project by seed companies and others will be acknowledged in the HDC Annual Reports, and at any associated meetings (unless you specifically wish us not to do so). We plan to issue email updates of results to project consortium members as testing progresses.

Ultimately the aim is to reduce the occurrence of disease outbreaks that are considered to have originated with the seed, for the benefit of the whole UK industry.

If you wish to participate in this project please contact me (01354 697215), Stuart Coutts (01694 781314) or Martin McPherson (01757 268275).

This letter is being sent out widely to the seed industry. If your company does not produce or market ornamental (bedding & pot plant) seed, then please treat is as 'for information only'.

Yours sincerely

T M O'Neill
Plant Pathologist

Distribution
Sakata Ornamentals UK Ltd
Tozer Seeds Ltd
Vale Royal Horticulture
Thompson & Morgan UK
Floranova
Moles Seeds
Syngenta Seeds Ltd
Nickerson Zwaan
Riijk Zwaan
Ball Colegrave
Flower Seeds Direct Ltd
Florensis
Warwicks
Ms Fay Richardson, Coletta & Tyson Ltd
Dr Ruth Finlay, HDC
Dr Martin McPherson, Stockbridge Technology Centre
Peter Byrne, W J Findon & Son Ltd
Stuart Coutts, Shropshire

Bacterial identification

Fatty Acid Profile Report from CSL on bacterial isolates from ornamentals seeds

ADAS isolates (6-11-06)

The closest matches are based on comparison with 2 libraries; TSBA40 is a broad spectrum aerobe library and NCPPB3 is based on plant pathogenic taxa and contains most strains in the NCPPB. Fatty acid profiling rarely proves identity but is often an accurate indicator at species level.

No. 1 *Antirrhinum* (CSL ref. 20620114)
TSBA40 – *Curtobacterium flaccumfaciens* (0.885)
NCPPB3 – *Curtobacterium lutem* (0.393)
No. 10 Wallflower – Cream (CSL ref. 20620115)
TSBA40 – *Bacillus megaterium* (0.783)
NCPPB3 – No match
No. 10 Wallflower – Yellow (CSL ref. 20620116)
TSBA40 – *Bacillus pumilus* (0.922)
NCPPB3 – No match
No. 14 Geranium – Yellow (CSL ref. 20620117)
TSBA40 – *Serratia liquefasciens* (0.788)
NCPPB3 – *Brenneria salicis* (0.534)
No. 14 Geranium – Cream (CSL ref. 20620118)
TSBA40 – *Lecleria adecarboxylata* (0.842)
NCPPB3 – *Pectobacterium cypripedii* (0.718)

STC isolates
No. 4 Geranium – White (CSL ref: 20704356)
Bacillus possibly *megaterium* (0.932)
No. 4 Geranium – Yellow (CSL ref: 20704357)
Bacillus possibly *cereus* (0.857)
No. 4 Geranium – White (CSL ref: 20704358)
Bacillus possibly pumilis (0.918)