Factsheet 13/09

Protected ornamentals

Project Horticulture LINK HL0166

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Guidelines for minimising latent grey mould (*Botrytis cinerea*) in cut flowers and pot plants

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Grey mould (*Botrytis cinerea*) can develop suddenly and unexpectedly on various cut flower and pot plant species throughout the supply chain resulting in significant wastage. These guidelines summarise current information on latent botrytis to help growers, packhouses and retailers reduce the risk of supply chain disruption due to the disease.

Action points

- Use seed shown to be free of botrytis, or treated to reduce seed-borne inoculum;
- Take cuttings from stock plants without visible botrytis;
- Practice good hygiene so as to reduce the levels of botrytis spores;
- Avoid high humidity periods of more than 3 hours (>85% if measured above the crop canopy);
- Avoid surface wetness of more than 1 hour (especially on flowers);
- Avoid condensation conditions;
- Ensure good air movement through the crop;
- Avoid sudden large drops in temperature;
- Be particularly vigilant with varieties known to be more susceptible;
- Harvest crop at the correct growth stage;
- Handle plants carefully to minimise damage and the risk of secondary botrytis infection;
- Use effective botrytis fungicides at key stages in crop production;
- Store plants in a cool environment and try to avoid holding them in stores for long periods of time.

Introduction

Latent botrytis refers to the symptomless occurrence of *Botrytis* species on and within plants. It is generally considered that much of the symptomatic botrytis that arises post-harvest originates from latent botrytis that was present on and within crops before harvest.

The annual wastage of cut flowers and pot plants in the UK resulting from symptomatic botrytis development from product rejection at nursery dispatch or the packhouse, in store or from returns to stores is estimated to be £24 million. Where a botrytis-susceptible species is used in a mixed bouquet, development of botrytis on that single species results in wastage of the whole bouquet (Figure 1). Products that have suffered from serious post-harvest botrytis wastage in recent years are listed in Table 1 (overleaf).

1 Rejection of gerbera due to botrytis results in wastage of other lines included in mixed bouquets

Table 1 Products that have suffered from serious post-harvest botrytis wastage in recent years

Pot Plants	Cut flowers
Cyclamen (Figure 2) Fuchsia Geranium Poinsettia Primula	Freesia (Figure 3) Gerbera Germini Lily* Lisianthus Peony* Rose Tulip*

*caused by a *Botrytis* species other than *B. cinerea.*

2 Development of botrytis on cyclamen frequently leads to post-harvest wastage

3 Infection by *B. cinerea* causes spotting on freesia

Research project

A four-year Horticulture LINK project (HL0166) was undertaken with the aim of reducing post-production losses from *B. cinerea* through a greater understanding of latency and by identifying key decision points in the supply chain that influence disease development. A summary of the findings on latent botrytis and practical guidelines resulting from the project are given below. The aim is to assist industry to assess potential risks and to adopt appropriate control measures where these may be necessary.

Summary of findings

- There is a risk of botrytis infection arising throughout the supply chain, originating with seed, cuttings and/or from air-borne spores, depending on the particular flower or pot plant species;
- A new molecular diagnostic test (TaqMan PCR) for latent *B. cinerea* is available as a tool for research use and possibly in future as a chargeable test for growers. The test is rapid, sensitive, specific and detects and quantifies the fungus simultaneously;
- Latent botrytis is common in many ornamentals and tends to increase in incidence and quantity during crop production;
- Only a small proportion of batches with latent botrytis develop symptoms;
- Batches can differ greatly in their incidence and quantity of latent botrytis varying, for example, with crop age, variety and nursery;
- Crop species differ in their relationship with *B. cinerea* and the most effective control measures are likely to differ also;
- Environmental and host factors influence botrytis progression; the interaction between botrytis form, quantity, host resistance and environment is complex and not fully understood.

Where does latent botrytis originate?

Many cut flowers and pot plants grown under protection in the UK carry symptomless (latent) *B. cinerea.* Spores of the fungus are relatively common in glasshouse air and these may account for the gradual increase with crop age in the proportion of plants in a crop in which latent *B. cinerea* can be detected. Further contamination and infection can accumulate during transport, storage and display from spores in the air. Additionally, symptomless infection may sometimes originate with the planting material, either seeds or cuttings. Latent *B. cinerea* was detected on cyclamen and polyanthus seed and in fuchsia and poinsettia cuttings. A list of ornamental species where seed-borne *B. cinerea* has been recorded is shown in Table 2.

Types of latent botrytis

Localised

B. cinerea can occur on plant material both externally and internally. Commonly it occurs on the outside of plant tissues as spores (conidia). These may remain viable for days to months, depending on the environmental conditions. Where the spore has not yet germinated and penetrated the plant cell wall, the association is generally termed contamination rather than infection. In the presence of surface moisture for as little as 1 hour (on flowers), or high humidity for 3 or more hours (> 85% if measured above the crop canopy), spores are likely to germinate and may penetrate cell walls. This may result in symptoms such as water-soaked petal spotting on cyclamen or gerbera (Figure 4), or flecking on poinsettia leaves within 24 hours. Where symptoms occur in such a short period of time, they are probably a plant response to the presence of the spore, rather than tissue breakdown due to colonisation by the fungus. If infection occurs but is contained by the plant's own defence mechanisms, there may be no visible symptom (a latent or quiescent infection). It should be noted that the term infection denotes that the fungus is within the plant; it does not necessarily mean that a plant is showing or will ever show disease symptoms.

Systemic

In the Horticulture LINK project, systemic infection by *B. cinerea* was recorded in cyclamen, poinsettia and primula. The fungus was detected in roots, stem, leaves and flowers and yet none of the tissues showed symptoms of infection. Often the same strain was present throughout a plant, with different strains in different plants. In a related study on lettuce plants with systemic infection, fungal strands (hyphae) were visible as a sparse network growing in the cortex of roots and stems and throughout leaves of plants, again without visible symptoms. Systemic infection was not detected in gerbera. The significance of symptomless systemic infection by *B. cinerea* in some hosts and not others is unknown. It is not known if such infections have any effect on the development of symptomatic botrytis.

Neither the widespread occurrence of latent *B. cinerea* in ornamentals, nor the existence of systemic symptomless infection in some species, was previously widely recognised. Both of these features could influence the efficacy of current disease control strategies and are likely to be the subject of future research. One immediate conclusion is that it appears that only a small proportion of latent infections by *B. cinerea* become symptomatic, otherwise one would expect to see botrytis symptoms in crops and post-harvest more widely.

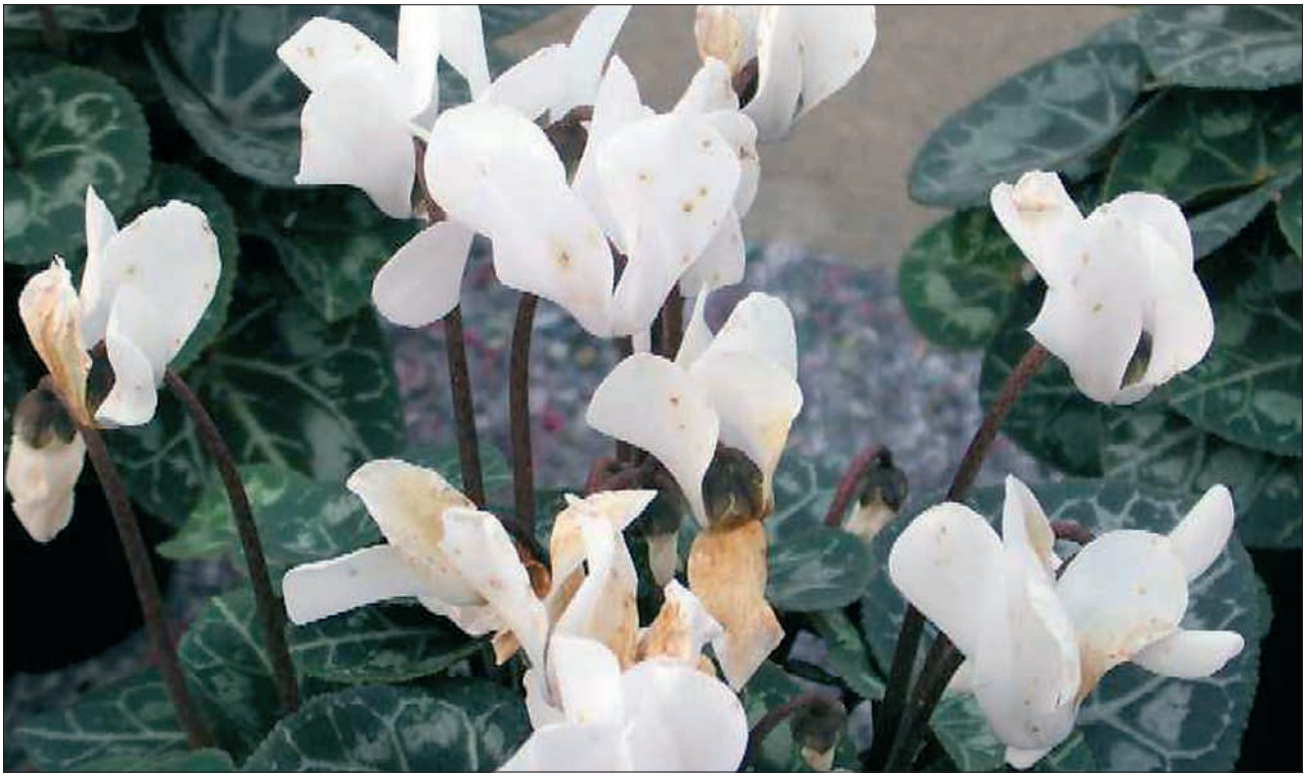
Table 2 Ornamental species where *Botrytis cinerea* has been detected on seed and/or cuttings

Ageratum	Matthiola
Antirrhinum	Nicotiana
Calendula	Pelargonium
Chrysanthemum	Phlox
Cyclamen	Primula
Dianthus	Senecio
Gerbera	Viola
Helianthus	Wallflower
Lobelia	Zinnia
Lupinus	

4 Some varieties of gerbera contaminated with botrytis spores develop petal spotting after just 1 hour of flower wetness

Activation of latent infections

The factors controlling latency are not clear. In green strawberry fruit, high water content in tissues was found to be important in the transition of latent *B. cinerea* to an aggressive phase. In the Horticulture LINK project on cut flowers and pot plants, the effects of environmental changes (eg increased humidity and surface wetness, temperature shock, watering pot plants prior to sleeving) on the quantity of latent botrytis in tissues and the development of symptoms, were relatively small and differed between species. The clearest effect was that petal spotting on flowers recently inoculated with *B. cinerea* conidia was greatly influenced by wetness duration (Figures 5 and 6).



5 Petal spotting and flower damage on cyclamen as a result of botrytis

6 Purple lesions on poinsettia following botrytis infection of wet bracts

Pathogen detection

Several methods are available to detect latent *B. cinerea* in plant material. At present they are used primarily as research tools, but there is continuing development in this area and they may become available as chargeable services in the future. Key features of each are summarised in Table 3.

Table 3 Comparison of the features of different tests for latent botrytis

Feature	Treatment and laboratory incubation*	TaqMan PCR**	Lateral flow device (LFD)***
Test duration	5–14 days	1–2 days	10 minutes
Output	Proportion of sample with viable botrytis (eg % leaves affected)	Quantity of *B. cinerea* DNA	Presence or absence of botrytis
Location of test	Laboratory	Laboratory or on-site	On-site
Amount of tissue tested	Leaf disc, whole leaf or flower petal	Large amounts are ground and a sub-sample of 0.1 g is processed	Leaf disc, whole leaf or flower petal
Specificity	All *Botrytis* species	*B. cinerea* (current test)	All/most *Botrytis* species
Potential problems	Underestimates infection due to suppression by other fungi. Limited quantification. Diagnostician required	Detects non-viable *B. cinerea*	Interference by some tissues (eg flowers). Limited quantification. Ungerminated spores not detected
Sensitivity	Medium	Very high	Medium
Approx. time (h) to process 40 samples	1.5	3.0	1.0
Approx. cost of consum-ables (£) per 40 samples	£1	£200	£200
Advantages	1 Use with surface sterilisation to discriminate internal and external botrytis 2 Large tissues (eg flower buds) can be tested	1 Very sensitive 2 Quantitative	1 Very quick 2 No specialist training required
Key uses	Research tool	Research tool. Can be used to check whether *B. cinerea* is associated with unusual symptoms	A quick on-site test to check a symptom for the presence of botrytis
Current status of test	Available as research tool (eg from ADAS)	Available as a research and diagnostic tool via Fera	Under development in UK. Available in USA for testing grape juice

* Tissue is killed by treatment with paraquat or by freezing and thawing, and then incubated at high relative humidity to encourage growth and sporulation of any *B. cinerea* on or within the tissue.

** DNA is extracted from the plant tissue and *B. cinerea* is detected and quantified using a polymerase chain reaction (PCR) test that detects a fragment of DNA unique to the fungus.

*** A monoclonal antibody within the LFD detects a specific enzyme secreted by species of *Botrytis.* An LFD test for symptomatic botrytis is now available from ForeSite Diagnostics; a test for latent botrytis is in development.

Assessing risks

A generalised check-list to help assess the risk of both visible and latent botrytis, during crop production and post-harvest is proposed (Table 4). Crops on a nursery and product received at a packhouse can be assessed for latent botrytis risk by scoring them using relevant categories, for example, on a 0–3 scale where 0=low risk and 3=high risk. Crops or products with a high score are more at risk of developing symptomatic botrytis along the supply chain. When five commercial cyclamen crops were assessed using this system, the rank order of crops closely matched that determined by quantification of latent botrytis within leaves using the TaqMan PCR test (Table 5 – overleaf).

Table 4 A check-list to assess the risk of infection by *B. cinerea* occurring during production of greenhouse crops of cut flowers and pot plants, and post-harvest

Factor	Low risk	High Risk
Nursery		
Glasshouse structure	Well heated, good air movement, good light	Unheated or poorly heated; little air movement; low light
Crop cultivation	'Hard' growth Crops grown in good light	'Soft' growth Crops grown over winter under low light
Host crop		
Crop species	Many foliage plants	Cyclamen, exacum, freesia, fuchsia, geranium, gerbera, lily, lisianthus, poinsettia, primula, rose
Variety		Some poinsettia, cyclamen and gerbera varieties
Growth stage	Active growth, no senescent tissue, not flowering	Young plant (seedling/cutting), flowering plant, mature plant
Tissue damage	No visible damage. Crop well spaced, handled and packed	Stock plants immediately after taking cuttings, or pinching (eg poinsettia) Cuttings during rooting (eg fuchsia) Senescent leaves present Mechanical damage during potting and spacing Spacing too tight
Crop production		
Evidence of botrytis during crop production	No visible botrytis in crop	Botrytis visible on plants, senescent tissue or debris
Occurrence of other problems during crop production	Healthy crop	Root rot, nutritional problem (eg calcium scorch), senescent tissue

Factor	Low risk	High Risk
Crop production continued...		
Harvest	Crop sold immediately at correct growth stage	Crop 'held' in production house, or cold-store
Botrytis		
Inoculum in air	No sporulating botrytis on crops or debris in or close to the house	Sporulating botrytis on crops or debris in or close to the house
Seed-borne	Not seed-borne, or seed treated against botrytis	Seed-borne (see Table 1 for at-risk species)
Cutting-borne	Stock plants grown in high-health quarantine conditions	Infected stock plants (eg fuchsia, poinsettia)
Environment		
Humidity	No condensation High VPD (>1.0 h Pa) Low RH (<85% above canopy) Forced ventilation	Risk of condensation occurring Low VPD High RH (>85% above canopy) Poor air movement
Surface wetness	None	More than 1 h (especially on flowers)
Temperature	No rapid temperature changes	Rapid changes resulting in condensation Temperature of 15–22°C (and light) for high conidial production by *B. cinerea*
Fungicide	Programme of effective fungicides at key timings in crop production	No effective botrytis fungicide treatments used during crop production
Post-harvest		
Humidity	Low RH No condensation No rapid temperature changes	Risk of flower spotting at high RH, or petal wetness >1h; risk of leaf infection if high RH >3 h
Temperature	Less botrytis spotting if product is stored cool	Risk of poinsettia bract rot with a temperature shock More gerbera flower spotting at warm temperature if the RH is high
Storage	Product processed and dispatched quickly	Product 'held' in store

Table 5 Assessment of post-harvest botrytis risk in five cyclamen crops according to crop production factors and quantification of latent *B. cinerea* in leaves from these crops

Risk assessment based on crop production factors*	Nursery				
	A	B	C	D	E
Use of botrytis fungicides	0	3	1	0	1
Botrytis visible at dispatch	2	2	3	0	1
Other disease in crop	1	0	2	0	2
Structure (glasshouse or tunnel)	0	2	0	0	0
Location of crop (bench or floor)	2	2	2	0	0
Cropping density	0	3	0	1	1
Heating system	2	2	0	0	0
Minimum temperature used	0	2	1	0	0
Air circulation fans used	0	2	0	2	0
Total risk score	7	18	9	3	5
Rank order (1=least risk, 5=highest risk)	3	5	4	1	2

Quantification of latent botrytis	Nursery				
	A	B	C	D	E
Mean quantity of latent *B. cinerea* (ng/g)	6.9	38.9	4.1	0.3	0.35
Rank order (1=least risk, 5=highest risk)	4	5	3	1	2

*Each factor assessed on a 0–3 scale, 0=low risk, 3=high risk.

Control of botrytis

The sudden development of visible botrytis symptoms after harvest may result from 1) recent infection by spores on the plant surface; 2) the re-activation of latent infections; or 3) the aggressive growth of previously symptomless systemic infection. Recommendations to prevent botrytis are generally based on avoiding the conditions favouring germination and infection by *B. cinerea* spores (Figure 7). While this will help towards reducing the risk of symptomatic botrytis developing, it is unlikely to prevent all botrytis outbreaks. More reliable control is likely to be achieved if the occurrence of latent infections is also minimised (see opposite).

At present, conditions that favour aggressive development of symptomless systemic or localised infections of *B. cinerea* are poorly understood. Further improved control of latent botrytis may become possible if these are better understood in any future work.

For a summary of environmental factors investigated in this project and their effect on development of latent botrytis in some cut flowers and pot plants, refer to Table 6 (overleaf).

The effect of fungicides on latent botrytis was not investigated in this project. Work elsewhere indicates fungicides may differ in their effectiveness against latent botrytis. Fungicides with systemic or translaminar activity are more likely to control latent infections than fungicides which act primarily by preventing spore germination on the leaf surface.

Minimising occurrence of latent botrytis

To minimise the accumulation of latent and visible botrytis during crop production and post-harvest, refer to the 'low risk' column in Table 3. Key recommendations, supported by results from HortLINK project HL0166 are:

- Use appropriate heating, ventilation and air movement to prevent condensation on plants during production of glasshouse ornamental crops.
- Prevent condensation on harvested product especially cut flowers by careful control of humidity (eg by dehumidification) during cooling, storage and transport.
- Promptly remove plants or product with symptomatic botrytis. There is some evidence that strains of *B. cinerea* sporulating on a host are more important as sources of further infection than strains from outside a glasshouse or from other crop species.
- For ornamental species that harbour long-term systemic infections (eg cyclamen, primula – Figure 8), control measures are required throughout the production cycle and should include seed as a possible infection source. See HDC Factsheets 23/02, 24/02 and 25/02 (see Further information).
- For ornamental cut flower species without long-term systemic infection (eg gerbera), control measures should be concentrated close to harvest and post-harvest.

7 A low relative humidity reduces the risk of botrytis leaf and stem rot in fuchsia

8 Control measures for botrytis on primula are required throughout the production cycle and should include seed as a possible infection source

Table 6 Summary of factors investigated and their effect on development of latent botrytis in some pot plants and cut flowers

Crop and factor	Detail*
Cyclamen	
High humidity	On inoculated plants, increased petal rot (100% vs 60% RH for 40 hours); no effect on leaf rot (80% and 100% RH for 5 days)
Temperature	On inoculated plants, no significant effect on symptoms (5, 10, 15°C for 5 days) A temperature drop increased quantity of latent botrytis in cyclamen leaves
Nursery	Levels of cyclamen leaf rot and petal spot differed between nurseries
Fuchsia	
Wetness and cold shock	Greater leaf botrytis incidence from wetness (24 h) combined with cold shock (4°C), than wetness alone
Transport	No effect of leaf wetness (72 hours), or cold shock (2–4°C), or refrigerated transport of cuttings from Portugal to UK, on leaf and stem botrytis on plants, or latent botrytis quantity in leaves
Poinsettia	
High humidity	No clear effect on bract lesions
Temperature	Bract lesions more severe after temperature shock at 15/5°C than at constant 15°C Greater botrytis 7 weeks after a temperature shock (5°C for 40 h) than at a constant temperature
Gerbera	
High humidity	Petal spotting greater at high RH (c. 100%) than low (50%)
Temperature	Petal spotting greater at 18°C than 4°C
Wetness	Petal spotting on inoculated flowers increased with wetness; only 1 h wetness or less is safe on a susceptible variety

*Plants uninoculated with *B. cinerea* except where indicated.

Further information

Other useful publications

- Control of grey mould *(Botrytis cinerea*) in container-grown ornamentals: unheated greenhouse crops. HDC Factsheet 23/02.
- Control of grey mould *(Botrytis cinerea*) in container-grown ornamentals: heated glasshouse crops. HDC Factsheet 24/02.
- Controlling humidity to minimise the incidence of grey mould (*Botrytis cinerea*) in container-grown ornamentals: heated glasshouse crops. HDC Factsheet 25/02.
- Use of chemical disinfectants in protected ornamental production. HDC Factsheet 15/05.
- Guidelines for the post-harvest handling of summer cut flowers and cut foliage. HDC Factsheet 02/06.
- Guidelines for post-harvest handling of cut lilies. HDC Factsheet 03/06.
- Guidelines for the post-harvest handling of cut tulips. HDC Factsheet 04/06.
- Guidelines on nursery hygiene for outdoor and protected ornamental crops. HDC Factsheet 10/07.
- HDC Project PC 234 examined factors affecting the quality of bedding plants during transport and distribution and included the monitoring of disease development including botrytis.
- Air movement in glasshouses – a grower's guide. HDC booklet. 2008.

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Additional information:

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