Infection of Commercial Hybrid Primula Seed by *Botrytis cinerea* and Latent Disease Spread Through the Plants

S. E. Barnes and M. W. Shaw

School of Plant Sciences, The University of Reading, Whiteknights, Reading, RG6 6AU, UK.

Current address of S. E. Barnes: School of Life and Environmental Sciences, University of Nottingham, University Park, Nottingham, NG7 2RD, UK.

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ABSTRACT

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Botrytis cinerea occurred commonly on cultivated *Primula xpolyantha* seed. The fungus was mostly on the outside of the seed but sometimes was present within the seed. The fungus frequently caused disease at maturity in plants grown from the seed, demonstrated by growing plants in a filtered airflow, isolated from other possible sources of infection.

Young, commercially produced *P. xpolyantha* plants frequently had symptomless *B. cinerea* infections spread throughout the plants for up to 3 months, with symptoms appearing only at flowering. Single genetic individuals of *B. cinerea*, as determined by DNA fingerprinting, often were dispersed widely throughout an apparently healthy plant. Plants could, however, contain more than one isolate.

Additional keywords: endophyte, gray mold, primrose, seed health, systemic.

Botrytis cinerea causes an economically important disease of container-grown ornamentals such as primula and cyclamen. This disease either reduces marketability of the plants or renders them unmarketable. The inoculum for the disease generally is thought to be spread by wind or splash dispersal of conidia onto above-ground plant parts. However, seedborne disease also may be important.

Seedborne *B. cinerea* is associated with a number of plant species, including *Stylosanthes* spp., a forage crop in sub-Saharan Africa (20), chickpea (6), flax and lettuce (13,14), lentil (18), and linseed (19). In linseed, 100% of the outer integument was found to be infected after 14 days of incubation (10). In total, *Botrytis* spp. have been isolated from seed of more than 50 flower, forage and vegetable species.

However, seedborne pathogens are relevant only if they can be transmitted to the plant growing from the seed (15). This is seen with infection of clover seed by *B. anthophila*, where the mycelium infects the seed and exists as a systemic pathogen (12). *B. cinerea* has been found to be transmitted from the infected seed to emerging seedlings in linseed and, after the seedling stage, few disease symptoms are seen until the right physiological stage of the crop is reached (9). *B. fabae* has been isolated from most parts of field bean seedlings grown from infected seed, although no obvious symptoms were present. The number of plants which later showed signs of sporulating pathogen was found to be temperature dependent (11). Some seedborne fungi such as *Alternaria* spp. are found to be associated with the plant throughout its growing season (14), and symptoms only appear once the environmental conditions are right or the host undergoes some physiological change.

Latent period has been defined as the period from infection until spore production (21). Latent infection by *B. cinerea* has been noted especially in grape (7), where prior infections of flowers

cause bunch rot later on in the season. Latency also has been found in raspberry (5), where disease of the fruit can result from germination of conidia on the stigma, with hyphae growing through the style. The same mechanism occurs in strawberry (5). Latent infection in parts of the plant other than the flower also has been observed in strawberry (4), where *B. cinerea* readily infects young leaves that then remain asymptomatic for up to 8 months until they senesce. In hybrid primula, we observed that inoculations with chlorate-resistant mutants produced no symptoms until flowering, regardless of when the inoculation was made (2).

The experiments reported here were undertaken with three aims: first, to determine if *B. cinerea* could be found in primula seed; second, to determine the prevalence of latent *B. cinerea* in healthy-looking hybrid primula plants; and third, to establish the relation between seedborne infection and epidemics in mature crops. An important intermediate goal was to determine whether latent infections of multiple plant parts were independent of each other or represented internal growth of one isolate.

MATERIALS AND METHODS

Surface sterilization method. To sterilize seed, they were soaked in a 20% solution of Domestos (0.1% hypochlorite) for 5 min. The effectiveness of this was tested on seed inoculated in two ways. In the first method, seed were mixed with dry conidia in a glass vial, then left for 1 h at room temperature; in the second method, seed were left to soak overnight in a suspension of 10^6 conidia per ml and dried in a flow of sterile air for 2 h. The seed were surface sterilized and plated onto 3% malt extract agar (MEA). In both cases, a chlorate-resistant mutant was used as a marked isolate in order to distinguish the isolate inoculated from any pre-existing infection.

The chlorate-resistant mutants were generated by inoculating *B. cinerea* isolates onto minimal medium, which contained ammonium nitrate at 0.1 g liter⁻¹, sucrose at 15 g liter⁻¹, and agar at 15 g liter⁻¹, and incubating for 3 days at 18°C. Growing cultures were inoculated onto the minimal medium amended with potassium chlorate (KClO₃) at 30 g liter⁻¹ and incubated at 18°C for up to 4 weeks,

Corresponding author: M. W. Shaw; E-mail address: m.w.shaw@reading.ac.uk

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during which the cultures were monitored for rapidly growing sectors. These sectors were transferred to fresh plates of chlorate-amended minimal media for 2 weeks at 18°C and from these to 3% MEA to allow growth and sporulation. Only isolates BC G (isolated from primula plants at a commercial grower in February 2000) and BC 133 (an isolate from primula plants grown at Reading University) showed the appropriate sectors. To ensure that the cultures were pathogenic, conidia were inoculated onto entire plants; sporulating pathogen was re-isolated and cultured on chlorate-amended minimal media plates to ensure that the dense growth still occurred.

Incidence of seed infected by *B. cinerea*. Batches of seed were obtained from commercial growers. In most cases, half the batch was surface sterilized in a 20% solution of Domestos for 5 min. The seed then were left to dry in a laminar flow cabinet. All seed then were placed on 3% MEA and incubated at 18°C in the dark for 6 to 8 days. Isolations were made from all seeds showing fungal growth.

A range of varieties of seed from four commercial producers and three retailers were examined. The seed had been stored for various lengths of time before tests; their true age was unknown.

Transmission of seedborne pathogen to growing plants. In order to determine if *B. cinerea* present in the seed would cause disease in mature plants, an isolation plant propagator was used (Burkhard Manufacturing Co. Ltd., Rickmansworth, Hertfordshire, UK). This is an apparatus designed to grow plants in an air stream free of fungal spores. Plants are grown in separate isolated pots located on trays, which have a reservoir of water in the bottom. Wicks, which run into each pot through the air pipe running up the center, are the only contact with the water. Filtered air is pumped through plastic piping into each tray. The air supply is shared between all the pots. The pots are covered with a transparent plastic dome, which contains two holes to allow the air to escape. The positive pressure of this air prevents contamination.

To determine if conidia were entering through the filter, three plates containing *Botrytis* spp.-selective medium (9) were placed in the pots for 8 h, or inverted over the exit air holes of the propagator, while open sporulating plates of *B. cinerea* were agitated for 15 min in front of the air intake.

In both experiments done in the isolation plant propagator, primula seed were sown in the pots and left to grow. The seed came from a batch of which 20% had been shown in the agar plate experiments to be infected after surface sterilization. In order to determine if *B. cinerea* in the soil contributed toward any final disease, 60 of the pots contained sterilized soil and 60 contained noninoculated, unsterilized soil. The soil was sterilized by autoclaving. In order to confirm that the conditions within the propagator were suitable for infection, 10 of the plants grown from seed were inoculated with a puff of conidia at the base of the plants, while the plants were at the two- to three-leaf stage. This inoculation was done by putting the tip of a Pasteur pipette with the bulb squeezed in a cryovial of spores, releasing the bulb to suck spores into the pipette, and then inoculating the plants with *B. cinerea* by sharply squeezing the bulb of the pipette close to the base of the plant. Ten more plants were inoculated 1 month later.

TABLE 1. Isolations of *Botrytis cinerea* from primula seed inoculated with dry conidia or a spore suspension after surface sterilization

Treatment ^y	Seed contaminated (%) ^z	
	Before sterilization	After sterilization
Seeds dusted with dry conidia	92 a	0 b
Seeds soaked overnight in a spore suspension	72 a	0 b
Control (noninoculated seeds)	40 a	4 b

^y Twenty-five seed were used for each test.

^z Numbers in the same row followed by a different letter are significantly different at $P = 0.01$.

The plants were removed from the plant propagator to be inoculated in the laboratory, then returned to the isolation plant propagator.

Changes in the experimental protocol became necessary in both experiments. First, some seed did not germinate, because the wicks did not work. These pots were equally from the sterilized and nonsterilized soil treatments. The dry pots were refilled with soil and new wicks put in place. Pregrown young plants, from a commercial grower, were planted in the pots. In the second experiment, the plant propagator had to be turned off for a period of 3 days because of greenhouse cleaning. By this stage, the top two trays had already been removed and only the bottom two trays remained, because the top plants flowered faster than the bottom plants. While the power was off, the entire propagator was sealed in plastic to prevent contamination.

At the end of the experiment, the plants were removed from the propagator and the presence of sporulating *B. cinerea* was recorded. The second leaf from the bottom was removed from each plant, surface sterilized, and plated on 3% MEA to check for *B. cinerea* infections not causing symptoms.

Position of latent disease within the plant. Fifty-five primula plants were received from a grower at the two- to four-leaf stage. To determine the level of latent infection, the leaves from five plants were harvested, surface sterilized, for 10 min in 20% Domestos, plated onto 3% MEA, and incubated at 18°C. This was repeated weekly for 11 weeks, until the plants were mature. The location of infection was determined in a further 48 mature primula plants which had been grown from seed in the glasshouse at Reading University under a normal light and temperature regime. These plants had necrosis on their lower leaves but were otherwise healthy. Forty-four of the plants were surface sterilized for 10 min in 20% Domestos. All plants were then aseptically dissected into top leaf tips; petiole of top leaves; bottom leaf tips; petiole of bottom leaves; old flowers; young flowers, and roots. Two leaf tips and two leaf bases were tested from each of the top and bottom leaves and one to two flowers were tested per plant. Approximately 5 to 10% of the total root system was tested per plant, depending on the overall root size. These pieces were plated onto 3% MEA and examined at intervals for sporulating colonies of *B. cinerea*.

DNA extractions. Isolates were grown on 3% MEA. A disk 5 mm in diameter was taken from the edge of the sporulating culture and added to 50 ml of 3% malt extract broth in a 100-ml conical flask. Flasks were incubated on a rotary shaker in a constant temperature growth chamber (Convion, Canada) for 5 days at 18°C with 12 h of light and 12 h of dark.

The flasks were removed and the culture filtered through two layers of sterilized muslin to retain the mycelium, which was washed twice with sterilized nanopure water. The mycelium was placed in a sterilized mortar and frozen in liquid nitrogen for 5 min. The mycelium was ground to a fine powder under liquid nitrogen with a sterilized mortar and pestle and placed in an Eppendorf tube. Eppendorf tubes were frozen at -20°C until the DNA was extracted, using a Qiagen DNeasy (Crawley, W. Sussex, UK) plant minikit according to the manufacturer's instructions.

DNA quantification. The extracted DNA, plus 1 µl of loading buffer, was run on a 1% agarose gel containing agarose in Tris-acetate-EDTA buffer with ethidium bromide. DNA concentrations were assessed visually under UV light and adjusted to approximately equal intensity by choosing an appropriate dilution.

Intersimple sequence repeat fingerprinting. Each reaction mix contained 2 µl of fungal DNA, 2 µl of NH₄, 0.6 µl of MgCl₂, 0.4 µl of dNTPs (from a stock solution prepared as 50 µl of each dNTP and 200 µl of sterile nanopure water), 0.3 µl of primer mix 888 (5' BDB[CA]₇ 3', where B is C, G, and T in equal frequency and D is G, T, and A in equal frequency; Genset Oligos, Genset Corp. Singapore), 0.1 µl of *Taq* DNA polymerase, and 14.6 µl of sterile nanopure water in a 50-µl Eppendorf tube. Two template

concentrations were used for each sample, differing by a factor of 10. Amplifications were done in a GeneAmp PCR system 9700 (Applied Biosystems, Warrington, UK) thermocycler. Initial denaturing was at 95°C for 3 min, followed by 30 cycles of denaturing at 94°C for 1 min, annealing at 55°C for 2 min, and extension at 72°C for 30 s. A final extension step was done at 72°C for 5 min, followed by storage at 4°C.

DNA band visualization. Precast acrylamide gels (Cleangel 48s, Pharmacia Biotech) were rehydrated, gel side down, in rehydration buffer (112 mM Tris acetate, pH 6.4) for 1 h. The gel was placed onto a premoistened flat bed gel runner, which had been cooled to 10°C. Two wicks, composed of five strips of filter paper cut into 4-by-25-cm strips, were soaked in 20 ml of electrode buffer (0.2 M Tris base, 0.2 M tricine, 0.55% [wt/vol] sodium dodecyl sulfate, pH 8.0) each and placed so that they overlaid the gel by 0.5 cm on either end.

Polymerase chain reaction (PCR) product (5 µl) was mixed with 2 µl of loading buffer (20% sucrose, 10% Ficoll, 0.05% bromophenol blue, 0.05% xylene cyanol, 1 mM EDTA, and 5 M urea) and loaded into the gel. Anode and cathode then were positioned 0.5 cm from the opposite side of each wick. The electrophoresis used a three-stage program: 20 min at 200 m Amax, 10 W max; 50 min at 380 m Amax, 20 W max; and 60 min at 450 m Amax, 20 W max.

Bands were detected by silver staining. Gels were fixed for 60 min in 250 ml of 10% acetic acid and then given four 2-min washes in nanopure water. They were silver stained for 30 min in 200 ml of freshly prepared 0.1% (wt/vol) silver nitrate with 200 µl of 40% (wt/vol) formaldehyde added immediately prior to use. Gels then were rinsed for 20 s in nanopure water and developed at 10°C in 200 ml of 2.5% sodium carbonate with 200 µl of 2% (wt/vol) sodium thiosulphate and 250 µl of formaldehyde added immediately prior to use. Development continued until the bands were clearly visible (approximately 5 min) and was stopped with 200 ml of 2% (wt/vol) glycine and 0.5% (wt/vol) EDTA-Na₂ for 10 min. The gel was impregnated in 250 ml of 5% (vol/vol) glycerol for 10 min in order to preserve it and air dried overnight.

Band patterns were compared by photocopying the gels onto transparency sheets, cutting out the separate pairs of lanes corresponding to different template concentrations, and overlaying them one at a time onto the original. Any differences in presence or position of the bands were noted. PCR, intersimple sequence repeat (iSSR) fingerprinting, and assignments to groups were repeated twice.

Statistical analysis. Counts were analyzed in Genstat (VSN International, Oxford UK) using generalized linear models with a logit link function. The association between isolate band patterns and plants was tested using a measure of association. If the frequency of band pattern *i* in the data was *p_i*, *r_j* isolations were made

from plant *j*, and *k_{ij}* isolates of type *i* were found in plant *j*, then the association was defined as

$$I = \sum_{i,j} \frac{k_{ij}}{r_j} \log(p_i)$$

Random assignments of the observed numbers of different types of isolates to the plants were made and the measure *I* calculated. Percentage points of the distribution of *I* were calculated from 100,000 simulated random assignments of band patterns to plants, keeping the total number of individuals with each pattern and the number of isolations from each plant constant.

RESULTS

Surface sterilization method. Surface sterilization removed *B. cinerea* from the seed coats of *Primula xpolyantha* seed inoculated by either technique (Table 1). A single seed, not inoculated, gave rise to a colony after sterilization.

Incidence of seed infection by *B. cinerea*. *B. cinerea* occurred externally on the *P. xpolyantha* seed obtained from most growers (Table 2). Seed from one grower also had a high incidence of *B. cinerea* that was not removed by surface sterilization.

Transmission of seedborne pathogen to growing *P. xpolyantha*. *B. cinerea* was isolated from mature leaves of primula, grown from noninoculated seed within the isolation plant propagator. Approximately half of the plants were infected although only 10 showed sporulation. The background level of seedborne *B. cinerea* was not known in this case. In plants initially grown outside the incubator and placed in the pots as plugs 4 months later, the proportion of leaves from which isolations could be made was significantly less ($\chi^2 = 6.3$, $P = 0.01$), with only 41% infected (Table 3). For plants grown from seed, there were no significant differences in infection incidence between inoculated and un-

TABLE 3. Number of mature primula plants found to harbor *Botrytis cinerea* when grown from seed in an isolation plant propagator or transferred there as plugs, in relation to soil treatment and seed inoculation

Plants from	Soil	Seed	Total sample ^y	Detection (%) ^z
Seed	Sterilized	Inoculated	11	73 a
		Not inoculated	66	59 a
	Not sterilized	Inoculated	25	60 a
		Not inoculated	64	70 a
Plugs	Not sterilized	Not inoculated	59	41 b

^y Both repeat experiments combined.

^z Numbers followed by the same letter do not differ significantly at $P = 0.05$, using a generalized linear model with a binomial error distribution and logit link function.

TABLE 2. Percentage of primula seed and seed batches from which *Botrytis cinerea* was isolated before and after surface sterilization in seed from various suppliers

Weeks from receipt	Seed producer	No. cultivars tested	Seed			
			Nonsterilized		Surface sterilized	
			No. cultivars infected	Seed infected (%) ^w	No. cultivars infected	Seed infected (%)
>24	Wholesale	17	7	31 ± 15 ^x	0	0
>24	Wholesale	3	3	33 ± 17 ^x	0	0
>24	Wholesale	3	1	17 ± 6 ^y	0	0
>24	Wholesale	4	1	17 ± 6 ^y	1	16 ± 6 ^y
1	Wholesale	5 ^z	1	30 ± 5 ^y	4	20 ± 0 ^{xy}
1	Retailer	3	3	24 ± 25 ^x	0	0
1	Retailer	2	1	8 ± 1 ^y	0	0
1	Retailer	2	2	22 ± 3 ^x	0	0

^w Mean proportion infected in batches with any *B. cinerea* detected.

^x Based on variation between cultivars.

^y Based on a binomial confidence limit for a sample size of 20.

^z Three of the samples plated out only after surface sterilization.

TABLE 4. Percentage of apparently healthy surface-sterilized parts of primula plants from which *Botrytis cinerea* could be isolated^w

	Age in weeks					Mean ^x
	4	5–7	9–11	12–14	15	
Plants sampled	14	15	15	15	20	...
Plants infected (%) ^y	43 a	93 b	100 b	73 c	100 b	...
Parts infected (%) ^z	100	67	73	70	81	...
Roots	43	43	87	33	80	56 a
Flowers	NP	NP	NP	33	45	38 a
Top leaf tip	43	53	67	40	70	54 a
Top leaf base	43	53	67	53	75	58 a
Bottom leaf tip	43	80	73	40	75	63 a
Bottom leaf base	43	80	73	53	60	65 a

^w No data were obtained in week 8; NP = organ not yet present in plants.
^x Numbers followed by the same letter are not significantly different at $P = 0.05$ using a generalized linear model of the counts, with binomial error distribution and a logit link function.
^y Percentage of plants tested in which at least one part was infected; numbers followed by different letters differ from the remainder at $P = 0.05$ according to χ^2 using a generalized linear model of the counts.
^z Percentage of parts infected in plants with at least one part infected.

TABLE 5. Percentage of apparently healthy parts of primula plants grown from seed in an open greenhouse from which *Botrytis cinerea* could be isolated^x

Plant part	Surface sterilized ^y	Nonsterilized ^z
Roots	71 a	50
Flowers	85 a	50
Top leaf tip	73 a	100
Top leaf base	58 a	100
Bottom leaf tip	79 a	75
Bottom leaf base	79 a	75

^x Forty-eight mature plants were tested, four without surface sterilization.
^y Numbers followed by the same letter are not significantly different at $P = 0.05$ according to χ^2 .
^z Sample size of four too small for meaningful statistical analysis between parts.

inoculated seed, or between sterilized and nonsterilized soil, or between repeat experiments.

Position of latent *B. cinerea* within *P. xpolyantha* plants. Plants 4 weeks of age and older showed a similar level of infection in all parts. There were fewer infected plants in the first batch tested ($P < 0.001$, using a generalized linear model), but there was no systematic trend over time (Table 4). The first batch of plants also showed a very strong association between one plant part and another: if a plant was infected anywhere, it was infected everywhere. This association was slightly less strong subsequently. Once the flowers began to develop, *B. cinerea* was found within them.

In mature plants, there were no significant differences in the proportions of different plant parts infected ($\chi^2 = 1.91$, 5 df, $P > 0.90$) (Table 5). All plants had some level of infection: 27% of the plants had all parts infected, 25% had 5/6 parts infected, 23% had 4/6 parts infected, 19% had 3/6 parts infected, and 6% had 2/6 parts infected. These proportions do not differ from the combinations expected by chance, given the average proportion infected, and testing the differences by χ^2 .

DNA band visualization. *P. xpolyantha* plants grown to flowering in an open glasshouse were dissected, isolations made from the various parts, and iSSR banding patterns visualized for as many isolates as possible (Fig. 1). In 9 of 10 cases, two or more isolations from distinct parts of a single plant yielded the same *B. cinerea* banding pattern (Table 6). Most plants harbored isolates with several distinct band patterns, and several band patterns occurred in isolates from more than one plant. However, multiple recoveries of individual isolate patterns from single plants were much commoner than expected by chance. Overall, the pattern was significantly different from a random assignment of isolates

Fig. 1. Intersimple-sequence repeat DNA band patterns of 23 isolates of *Botrytis cinerea* from different parts of five flowering but symptomless plants from a crop grown in an open glasshouse at Reading from commercial plug plants. Each isolate is represented in a pair of adjacent lanes, with polymerase chain reaction done from the same extract at standard and 1/10 dilutions. Numbers distinguish the plants from which isolates came. Letters represent plant parts: F, flower; TLT, top leaf tip; TLB, top leaf base; BLT, bottom leaf tip; BLB, bottom leaf base; R, root.

to plants ($P \leq 0.0001$, using the randomization test described). In plants 1 and 8, *B. cinerea* was recovered and fingerprinted successfully from throughout the plant, and all isolates were extremely similar. By contrast, isolates recovered from plant 4 were all distinct from one another, suggesting multiple independent infections.

DISCUSSION

B. cinerea was associated with *P. xpolyantha* seed in these experiments and surface sterilization was able to remove conidia from the seed coat with high efficiency. This suggests that the *B. cinerea* isolated from seed once they were surface sterilized was extremely likely to be from inside the seed. Visualization experiments will be needed to determine the position of this pathogen within the seed. In linseed, *B. cinerea* was associated with the outer integument (10) but primula seed are far smaller and *B. cinerea* mycelium might be less localized within the seed.

The proportion of *P. xpolyantha* seed infected was moderate, with a maximum of 22%. This is higher than in faba bean, where

TABLE 6. Grouping of intersimple sequence repeat fingerprinting band patterns of isolates from different primula plant parts^z

Organ	Plant number									
	1	2	3	4	5	6	7	8	9	10
Flowers	1 a	u	...	...	8 b	...	7	8 b	...	...
Top leaf tip	1 a	1 b	1 e	u	u	6 a	7	8 a	9	10 a
Top leaf base	1 b	1 d	3 b	7 a	...	...	7	...	9	10 b
Bottom leaf tip	1 a	3 a	3 b	1 c	8 b	6 a	...	8 a	...	10 a
Bottom leaf base	1 a	3 a	3 b	u	u	6 b	u	...	9	u
Roots	1 c	1 e	...	8 b	8 b	8 c	...	8 c	10 b	...

^z Numbers represent different patterns predominant in particular plants; letters which differ denote one- or two-band discrepancies in weaker bands; u indicates a unique pattern; and ... indicates inadequate or no amplification occurred from the extract or no isolation was made.

up to 8% of the seed have been found to be infected with *B. fabae* (11). At this percentage, seed transmission was considered to be important in initiating disease epidemics (11). In flax, much of the seed infection was superficial, with internal mycelium occurring only occasionally (15). Our studies suggest that distinct infection types may exist because some seed batches had no internal infection whereas, in others, a very large proportion was in-eradicable (Table 2, lines 4 and 5).

A large number of the primula seed were found to have *Penicillium* sp. and *Rhizopus* sp. associated with them, along with bacterial contaminants. In other host species, such as a *Stylosanthes* sp., pretreatment of the seed with 1% sodium hypochlorite has been found to increase the amount of *B. cinerea* isolated because there is a decrease in the development of *Penicillium* sp. and *Rhizopus* sp., which hinder the development of slower-growing fungi (20). This may explain why we found a greater proportion of plants infected with *B. cinerea* in the plant propagator, where seed were sterilized, than expected from isolations from the unsterilized seed.

Using the isolation plant propagator, it was possible to demonstrate that *B. cinerea* was transmitted to growing *P. xpolyantha* plants from the seed, because no other source of infection was available. Sterilization of the soil did not decrease the amount of disease seen; therefore, soil could not be considered a major source of inoculum. *B. cinerea* has been shown to be transmitted to seedlings from infected seed; for example, in linseed (17), chickpea (6), and lentil (18). However, it has not previously been shown to be able to grow systemically within a plant without symptoms until the plant is fully mature.

B. cinerea was found throughout *P. xpolyantha* plants as a latent infection at a high frequency in 4-week-old seedlings; frequencies of infection rose slightly in the next week and remained high until the plants had reached maturity. In the initial material, it was particularly noteworthy that, if a seedling was infected at all, all parts were infected. Neither observation is consistent with epidemiology involving only accumulation of individual conidial infections. Mature plant parts examined for latent *B. cinerea* showed a high level of disease in the roots, which is hard to explain other than by spread from elsewhere in the plant. Blakeman (3) found infection of lettuce roots by a specialized form of *B. cinerea*, which only attacked the remainder of the plant as it senesced. This differs from the present case in the localization of the infection. In this context, it is notable that isolates giving band pattern 8 appeared to be preferentially associated with roots in the plants from which isolates were taken for fingerprinting (Table 6). *B. cinerea* has been shown to grow systemically through the styles of various flower parts, such as black currant flowers (16), and within the vascular system of pear flowers (8), but it has not previously been shown to grow systemically through a growing herbaceous plant.

B. cinerea is well known to form latent infections within plants but this mainly is considered to be more in the form of a quiescent infection (1,3), where the fungus germinates on the plant surface and remains dormant just under the epidermal cells. In our case,

B. cinerea was spread throughout *P. xpolyantha* plants and results using both genetic fingerprinting and the isolation propagator suggested that fully systemic infection by single isolates might be common. These results strongly suggest that *B. cinerea* frequently grows within *P. xpolyantha* as a systemic infection. Infections via conidia from outside sources would explain the remaining sporadic infections in systemically infected plants. Further work is needed to determine whether systemic infection occurs in other species; our preliminary work suggests that *B. cinerea* often can be recovered from all parts of healthy commercial hybrid *Cyclamen* plants, also in the primulaceae.

Systemic *B. cinerea* infection of commercial *P. xpolyantha* may explain why the disease is sometimes hard to control with fungicides. This is observed in other species also: for example, even with very regular sprays of the fungicide captan, strawberry yield losses of 10 to 15% still occur due to *B. cinerea* infections (13). It is possible that partially or fully systemic infections contribute significantly to losses in this or analogous cases.

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