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Characteristics of *Pestalotiopsis* associated with hardy ornamental plants in the UK

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Abstract

Pestalotiopsis isolates obtained from the foliage, stem-base and roots of hardy ornamentals grown on commercial nurseries in the UK were identified and characterised according to pathogenicity and colony morphology. All 18 isolates were identified as *Pestalotiopsis sydowiana* on the basis of conidia morphology, and confirmation of identification was made by experts at CABI Bioscience. Isolates were pathogenic on the host from which originally isolated. Typical symptoms included foliar browning of foliage and stems, and the presence of black or greenish-black acervuli on diseased tissue. Isolates were not host specific and infected other species of hardy ornamentals. Three colony types on potato dextrose agar were distinguished according to colour and production of acervuli by individual isolates.

Three selected isolates of *P. sydowiana* were characterised by examining the effects of growth media, temperature, pH, and water potential on hyphal extension. Isolates grew well on commonly used growth media, including PDA, Sabouraud dextrose agar (SDA), V8 juice agar (V8), malt extract agar (MEA) and Czapek Dox agar (CDA). The optimum temperature for growth on PDA was in the range 20–25 °C, with little or no growth occurring below 5 °C or above 30 °C. Hyphal extension occurred over a pH range between 2.6–8.6, with optimum values occurring at pH 5.5. In general, decreases in osmotic and matric potential caused a reduction in growth. Hyphal extension on media adjusted osmotically as NaCl ceased between −9.9 and −10.5 MPa. Isolates were more tolerant of osmotic than matric potential, with no growth occurring at −6.5 MPa on media adjusted with polyethylene glycol.

Introduction

Changes in the production methods for hardy ornamentals have resulted in increasing problems with disease. After recognised major pathogens are brought under control by improved management techniques and the use of effective but highly specific fungicides, plants may become more vulnerable to attack by less competitive pathogens which have previously been considered weak or secondary. *Pestalotiopsis* spp. were formerly regarded as weak, opportunistic pathogens which caused little damage to hardy ornamentals (Pirone, 1978; Coyier, 1986). However, in the past 10 years these pathogens have been increasingly reported to cause widespread damage to a number of container-grown plant species. During recent seasons, an increasing number of damaging infections of *Calluna vulgaris*, *Erica* spp., *Rhododendron* and conifers by *Pestalotiopsis* spp. were found on plant samples submitted by growers to the SAC Crop Health Centre. Serious damage has been seen on cuttings, potted-on plants and stock plants. On *C. vulgaris*,

Table 1. Origins of isolates of *Pestalotiopsis sydowiana* and pathogenicity on hosts from which originally isolated

Isolate	Host	Plant part	Origin	IMI[1] number	Disease severity[2]
P1	*Calluna vulgaris*	Foliage	Ford, Scotland	358103	2.8
P2	*Chamaecyparis lawsoniana*	Foliage	Lanark, Scotland	356304	2.1
P3	*Rhododendron*	Foliage	Braevallich, Scotland	356305	2.5
P4	*Rhododendron* cv. Princess Anne	Foliage	Braevallich, Scotland	—	2.3
P5	*Erica* sp.	Foliage	Maghera, N. Ireland	356306	2.8
P6	*Rhododendron*	Foliage	Perth, Scotland	356308	1.9
P7	*Rhododendron*	Foliage	Taynuilt, Scotland	356309	2.5
P8	*Erica carnea*	Foliage	Lochgilphead, Scotland	358104	2.7
P9	*Rhododendron* cv. Coral Velvet	Stem-base	Haywards Heath, England	356311	2.5
P10	*Rhododendron* cv. Golden Torch	Foliage	Dalmally, Scotland	356312	2.5
P11	*Rhododendron* cv. Hachmanns Rosita	Roots	Droitwich, England	358105	2.7
P12	*Rhododendron* cv. Cowslip	Roots	Braevallich, Scotland	358106	2.3
P13	*Aralia elata*	Foliage	Edinburgh, Scotland	356310	2.5
P14	*Juniperus*	Foliage	Edinburgh, Scotland	358107	2.6
P15	*Euonymous fortunei*	Foliage	Droitwich, England	358102	1.8
P16	*Pieris* sp.	Stem-base	Barguillean, Scotland	—	2.0
P17	*Rhododendron luteum*	Roots	Chobham, England	—	1.7
P18	*Erica aborea*	Roots/stem	Lochgilphead, Scotland	—	2.7

[1]CABI Bioscience (former International Mycological Institute).
[2]Mean disease severity of 15 cuttings, 4 weeks after inoculation, where 0 = no foliar browning, 1 = <50% foliar browning, 2 = >50% foliar browning and 3 = death of cutting.

Erica spp. and conifers, the symptoms are necrosis and death of the foliage, with extensive acervuli production on affected parts appearing as tiny black or brown spots (Hopkins, 1996). On *Rhododendron*, disease was evident as brown/bleached lesions at various points on the stems, but sometimes there was a more general rotting of the roots and stem base (Hopkins, 1996; Hopkins and McQuilken, 1997). Several species of *Pestalotiopsis* may be associated with damage, but *P. guepini* is believed to be most common (Mordue, 1971).

Pestalotiopsis spp. pose a threat to hardy ornamental production by causing plant losses, reduced plant quality and disruption of production schedules. It is prudent to obtain information on the identity and biology of the main species of *Pestalotiopsis* associated with hardy ornamentals so that effective control can be implemented. The objectives of this investigation were to: (i) identify and determine the cultural characteristics of the main species of *Pestalotiopsis* affecting hardy ornamentals; (ii) establish the pathogenicity and host range of the most consistently isolated species; (iii) examine the effect of environmental factors (temperature, pH and water potential) on the growth of the pathogen so facilitating prediction of its likely behaviour in nature.

Materials and methods

Collection and isolation

Hardy ornamental plants with typical symptoms of Pestalotiopsis disease were collected from several commercial nurseries throughout the UK (Table 1). Segments of diseased plant tissue (3–5 mm^2) were cut, surface-sterilised in 10% (v/v) sodium hypochlorite for 5–10 s and rinsed in three changes of sterile distilled water. Surface-sterilised segments of tissue were blot-dried on sterile filter paper and placed on Oxoid potato dextrose agar (PDA; Unipath, Basingstoke, Hants, UK) modified to contain 100 $\mu g\ ml^{-1}$ of both erythromycin and streptomycin (PDAES). Plates were incubated at 20 °C. After 3–5 days, hyphal tips of mycelia growing from segments were transferred to single Petri dishes of PDAES in order to maintain pure cultures.

Identification

Identification of *Pestalotiopsis* isolates (Table 1) was based on morphological characteristics of conidia (Sutton, 1980). Conidial suspensions of each isolate were prepared by flooding 21-day-old PDA Petri dish

cultures with sterile distilled water and gently scraping the colony surfaces with a sterile bent glass rod. Conidia were diluted to a final concentration of $1–3 \times 10^6$ conidia ml^{-1}. Using a light microscope ($\times 400$ magnification) fitted with an eye-piece graticule, the following were measured for each of 30–50 conidia: (1) length and width; (2) length of median cells; (3) length of apical and basal appendages. Number of cells, appendages and the colour of the apical, basal and the two superior median cells were also recorded for each conidium. Definitive identification of isolates was provided by experts at CABI Bioscience (Egham, Surrey, UK).

Pathogenicity testing and host range

Isolates of *Pestalotiopsis* were tested for pathogenicity on the host from which they were originally isolated. Fifteen unrooted cuttings of each host were planted in trays ($23 \times 18 \times 6$ cm) containing propagation compost (Bulrush Peat Co. Ltd., Magherafelt, Co. Londonderry, N. Ireland). Immediately after planting, cuttings were lightly dusted with carborundum powder and artificially inoculated by brushing individual cuttings with a spore suspension ($1–3 \times 10^6$ conidia ml^{-1}) of each isolate. Preliminary experiments indicated that prior wounding of plant tissue by dusting with carborundum was necessary to establish infection. Non-inoculated cuttings served as controls. Trays of cuttings were covered with white polythene (20 μm thick) on a gravel bench in a glasshouse maintained at 12–24 °C. Cuttings were examined twice weekly for 4 weeks for foliar browning and the presence of black spore masses of the pathogen. To confirm infection and fulfill Koch's postulates, small pieces of diseased tissue were placed on PDAES and observed for colonies typical of the pathogen. Foliage of each cutting was assessed after 4 weeks for disease severity, using a severity scale of 0–3, where 0 = no foliar browning, 1 = <50% foliar browning, 2 = >50% foliar browning and 3 = death of cutting.

The host range of isolates P1, P2, P5 and P10, all from different host species, was determined on unrooted cuttings as described before.

Cultural characteristics

Five replicate 9 cm diameter Petri dishes containing PDA (15 ml) were inoculated centrally with an agar disc (3 mm diameter) of each isolate of *Pestalotiopsis*, cut from the edge of an actively growing colony on PDA. Cultures were incubated in the dark at 20 °C and cultural morphology was examined after 21 days. Colony colours of individual isolates were defined according to Raynor (1970), and the production of acervuli was assessed visually.

Effect of growth medium, temperature, pH and water potential

To determine the effect of growth medium, temperature, pH and water potential on the development of *Pestalotiopsis*, hyphal extension rate was examined. Seven agar media were used: PDA, Oxoid Czapek Dox agar (CDA), Oxoid Sabouraud dextrose agar (SDA), V8 juice agar (V8; 200 ml Campbell's V8 vegetable juice (King's Lynn, Norfolk, UK), 20 g Oxoid Technical agar No. 3 per litre tap water), Oxoid malt extract agar (MEA), Oxoid cornmeal agar (CMA) and tap water agar (TWA; 20 g Technical agar No. 3 per litre tap water). Sterile media were adjusted to pH 5.6 with 1 M NaOH or HCl and 15 ml dispensed into 9 cm diameter Petri dishes.

The effect of temperature was studied by incubating inoculated plates of PDA in the dark at 5, 15, 20, 25, 30 and 35 °C. To examine the effects of medium pH, batches of sterile double-strength PDA were adjusted with 1 M NaOH or HCl to the required pH and an equal volume of buffer was added to give the correct concentration of medium. The pH was maintained over the ranges of 2–7 and 7.2–9.2 with citrate phosphate (0.05 M citric acid, 0.1 M $Na_2HPO_4 \cdot 7H_2O$) and Tris (hydroxymethyl) aminoethane (0.1 M Tris, 0.1 M HCl) buffers, respectively (Gomori, 1955).

To study the effect of osmotic potential, PDA was adjusted osmotically over the range -0.3 to -10.3 MPa with NaCl (Lang, 1967). The total water potential was the sum of the water potential of PDA (-0.3 MPa) and the osmotic potential of added osmotica. To study the effect of matric potential, PDA was adjusted matrically with polyethylene glycol (PEG 6000) (Steuter et al., 1981). PEG 6000 (g kg^{-1} liquid) of different concentrations (equivalent to -0.8, -1.3, -2.1, -4.8, -6.5, -7.8, -9.1 MPa) for incubation temperatures of 20 °C were determined from a formula derived by Michel and Kaufman (1973). Concentrations of PEG 6000 were autoclaved separately, cooled to 50 °C and added to PDA to give the required matric potentials. Sterile medium (15 ml) of PDA/PEG 6000 at each matric potential was dispensed into 9 cm diameter Petri dishes. Boiled autoclaved 9 cm diameter

cellophane discs (PT 600; British Cellophane Co.) were placed on the matrically adjusted media to provide a suitable platform for growth because the PDA/PEG 6000 did not solidify completely below −1.5 MPa.

Assessment of hyphal extension rate

To measure hyphal extension rate, agar discs (3 mm diameter) cut from the edge of an actively growing colony on PDA, were placed on the centre of agar plates, and incubated in the dark at 20 or 22 °C. Five replicate plates were inoculated with each isolate and hyphal extension rate was measured daily between 4 and 6 days by measuring the increase in colony diameter.

Statistical analyses

For most experiments, data were assessed using an analysis of variance (ANOVA) and treatment means were compared with the least significant difference (LSD) at a probability of 5%. Analysis of disease severity data was carried out on square root transformed data. Data on spore measurements are presented as means ± standard error (SE).

Results

Isolation and identification

Eighteen isolates of *Pestalotiopsis* were isolated from a range of diseased container-grown hardy ornamentals collected from nurseries in England, Scotland and Northern Ireland (Table 1). Isolates were obtained from the foliage, stem-base and roots of infected plants. All isolates were identified as *Pestalotiopsis sydowiana* (Bresad.) B. Sutton (Sutton, 1961) on the basis of conidia morphology. Confirmation of identification was made by CABI Bioscience, and representative cultures of some of the isolates were lodged in the culture collection for future reference.

All isolates of *P. sydowiana* had 5-celled conidia. Apical and basal cells of all isolates were hyaline, while the 2 superior median cells and inferior one were dark brown and light brown, respectively. Measurements of conidia revealed few differences between isolates. Conidia varied from 22.2–24.8 μm mean length (Table 2) and 6.1–7.3 μm mean width. Median cells were 15.7–17.9 μm mean length. Basal appendages were hyaline, straight or slightly curved and 2.5–7.0 μm mean length. Numbers of apical appendages ranged from one to five, with three being the most common; they were 15.5–29.4 μm mean length.

Table 2. Characteristics of conidia of *P. sydowiana* isolates

Isolate	Conidia size (μm)		Length of median cells (μm)	Appendage length (μm)		No. of apical appendages (range)
	Length	Width		Apical	Basal	
P1	24.8 ± 2.2[1]	6.7 ± 0.5	17.9 ± 1.6	22.6 ± 2.2	3.8 ± 0.5	2–3
P2	23.8 ± 1.6	6.5 ± 0.6	17.2 ± 1.1	39.4 ± 1.8	7.0 ± 1.1	1–3
P3	23.4 ± 2.2	7.0 ± 1.2	16.8 ± 1.7	26.7 ± 3.0	4.9 ± 0.7	2–3
P4	23.4 ± 2.2	6.5 ± 0.6	16.9 ± 1.1	24.5 ± 2.2	4.1 ± 0.4	2–4
P5	23.0 ± 2.2	6.7 ± 1.1	17.0 ± 2.2	22.8 ± 1.6	4.0 ± 0.3	2–4
P6	22.2 ± 1.6	6.8 ± 0.6	15.7 ± 1.1	23.6 ± 2.7	2.8 ± 0.7	2–4
P7	22.5 ± 2.2	6.4 ± 0.6	16.2 ± 1.8	20.5 ± 1.3	4.1 ± 0.8	2–4
P8	23.5 ± 2.6	6.3 ± 0.6	16.7 ± 2.3	23.1 ± 1.2	4.4 ± 1.0	3–4
P9	22.6 ± 2.1	6.5 ± 0.5	16.4 ± 1.6	24.2 ± 2.6	5.1 ± 0.5	2–3
P10	23.0 ± 2.8	7.0 ± 0.6	16.0 ± 2.2	19.3 ± 0.8	4.1 ± 0.6	2–4
P11	21.9 ± 1.6	6.4 ± 0.5	16.2 ± 1.1	18.1 ± 1.1	5.3 ± 0.5	2–4
P12	22.6 ± 1.1	6.1 ± 0.6	16.6 ± 0.6	18.9 ± 2.3	4.0 ± 0.8	2–4
P13	22.2 ± 2.2	6.4 ± 0.5	16.3 ± 1.6	19.0 ± 0.5	2.5 ± 0.4	2–4
P14	22.4 ± 2.2	6.5 ± 0.4	14.3 ± 1.8	15.5 ± 0.8	4.3 ± 0.5	2–4
P15	24.0 ± 2.2	7.0 ± 0.6	17.0 ± 1.7	24.3 ± 2.4	4.4 ± 0.3	2–4
P16	22.4 ± 1.0	7.1 ± 0.5	16.6 ± 1.1	22.4 ± 2.7	3.2 ± 0.5	2–3
P17	23.6 ± 1.6	7.3 ± 1.1	16.7 ± 1.1	24.4 ± 3.1	5.0 ± 1.1	2–4
P18	23.8 ± 1.6	7.0 ± 0.6	16.8 ± 1.0	23.6 ± 2.5	5.8 ± 0.7	2–4

[1]Each value is the mean ± SE from measurements of 30–50 conidia.

Table 3. Host range of four isolates of *P. sydowiana* on selected ericaceous plants and conifers 4 weeks after inoculation

Isolate[1]	Disease severity (0–3)[2]				
	E. cinerea	*C. vulgaris*	*Rhododendron*	*C. lawsoniana*	*C. leylandii*
P1	3.0 (1.73)[3]	2.8 (1.69)	2.2 (1.45)	2.5 (1.57)	1.9 (1.38)
P2	2.6 (1.61)	2.8 (1.66)	2.1 (1.44)	2.8 (1.68)	2.7 (1.64)
P5	2.8 (1.67)	2.9 (1.68)	2.3 (1.53)	2.5 (1.36)	2.3 (1.23)
P10	2.4 (1.56)	1.9 (1.35)	2.5 (1.59)	2.2 (1.49)	2.1 (1.47)
LSD ($P = 0.05$)[4]	(0.121)				

[1]P1, P2, P5 and P10 were originally isolated from *C. vulgaris*, *C. lawsoniana*, *Erica* spp. and *Rhododendron* (see Table 1 for details).
[2]Disease severity 4 weeks after inoculation where 0 = no foliar browning, 1 = < 50% foliar browning, 2 = >50% foliar browning and 3 = death of cutting.
[3]Mean disease severity of 15 cuttings; values in parentheses are square root transformed data.
[4]LSD is the least significant difference at a probability of 5% ($P = 0.05$).

Pathogenicity testing and host range

All isolates of *P. sydowiana* were pathogenic on the host from which they were originally isolated (Table 1). All inoculated cuttings developed symptoms similar to those on the original plants collected from nurseries. Typical symptoms, 4 weeks after inoculation, included foliar browning of leaves and stems, and the presence of black or greenish-black acervuli on diseased tissue. Some isolates caused death of cuttings. Koch's postulates were fulfilled for all of the isolates by re-isolation on PDA.

Isolates P1, P2, P5 and P10, all from different host species, were pathogenic on cuttings of *Calluna vulgaris* (cv. Kinlochruel), *Erica cinerea* (cv. Lyonesse), *Rhododendron* (cv. Curlew), *Chamaecyparis lawsoniana* and *Cupressocyparis leylandii* (Table 3). All inoculated cuttings developed typical symptoms within 4 weeks of inoculation, but significant differences ($P = 0.05$) were found in the aggressiveness of the isolates tested. Isolates P1 and P5 were considerably more pathogenic on *C. vulgaris* and *E. cinerea* than on *Rhododendron* or the two species of conifer.

Cultural characteristics

The isolates of *P. sydowiana* could be clearly separated by their colour on PDA into three main groups. The first, pale buff from above and either pale buff or pale luteous from below, contained the isolates P1, P2, P4, P5, P6, P8, P11, P12, P16, P17, P18 and P19. Acervuli were black, sparse to abundant and scattered over the surface of the colony. The second, pale luteous from above and luteous or pale luteous from below, contained the isolates P3, P7, P9 and P13. Acervuli were black and sparse, except for P7, which produced abundant greenish-black acervuli. The third group contained isolates P10, P14 and P15, which were pale salmon from above and below. Acervuli were black and abundant. Isolate P14 produced acervuli in concentric rings. One isolate was selected from each group for media, temperature, pH and water potential studies.

Effect of media

All three isolates (P5, P7 and P10) of *P. sydowiana* grew on the seven different agar media tested (Table 4). Hyphal extension rates ranged between 4.0–8.0 mm day^{-1} with relatively small but significant differences between isolates and media. Extension rates of all three isolates tended to be lower on CMA (4.0–6.4 mm day^{-1}) and TWA (4.4–5.8 mm day^{-1}) compared with the other five media.

Effect of temperature and pH

Temperature significantly ($P = 0.05$) affected the hyphal extension rates of all three isolates of *P. sydowiana* (Table 5). P7 grew over the range 5–30 °C, with a maximum extension rate (5.4 mm day^{-1}) at 25 °C. Both P5 and P10 grew between 10–30 °C, with maximum rates of approximately 4.5–5.5 mm day^{-1}

Table 4. Effect of different growth media on radial hyphal extension rate (mm day^{-1}) of three isolates of *P. sydowiana* at 22 °C

Isolate	Media						
	PDA[1]	MEA	CMA	CDA	SDA	V8	TWA
P5	5.6[2]	7.6	4.0	5.8	8.0	7.2	5.4
P7	7.6	6.8	5.4	5.2	6.6	7.0	4.4
P10	7.4	6.4	6.4	7.4	7.6	7.2	5.8
LSD ($P = 0.05$)[3]	0.34						

[1]See materials and methods for media abbreviations.
[2]Values are mean hyphal extension rates (mm day^{-1}) of five replicates measured between 4 and 6 days.
[3]LSD is the least significant difference at a probability of 5% ($P = 0.05$).

Table 5. Effect of temperature on radial hyphal extension rate (mm day^{-1}) of three isolates of *P. sydowiana* on PDA

Isolate	Temperature (°C)					
	5	10	15	20	25	30
P5	0[1]	2.6	3.2	4.5	5.2	0.6
P7	0.5	2.8	3.3	4.6	5.4	0.3
P10	0	2.4	3.1	5.1	5.1	2.8
LSD ($P = 0.05$)[2]	0.43					

[1]Values are mean hyphal extension rates (mm day^{-1}) of five replicates measured between 4 and 6 days.
[2]LSD is the least significant difference at a probability of 5% ($P = 0.05$). (There was no growth of any isolate at 35 °C.)

Table 6. Effect of pH on radial hyphal extension rate (mm day^{-1}) of three isolates of *P. sydowiana* on PDA at 20 °C

Isolate	pH								
	2.6	3.8	5.0	5.5	6.3	7.0	7.6	8.0	8.6
P5	4.1[1]	4.9	5.4	6.5	3.6	3.5	3.0	2.7	0.8
P7	3.7	3.8	4.5	5.6	2.6	2.2	1.8	1.2	0.6
P10	1.4	4.6	4.7	6.2	2.2	2.0	2.9	1.4	0.6
LSD ($P = 0.05$)[2]	0.68								

[1]Values are mean hyphal extension rates (mm day^{-1}) of five replicates measured between 4 and 6 days.
[2]LSD is the least significant difference at a probability of 5% ($P = 0.05$). (There was no growth of any isolate at pH 9.0.)

occurring at 20–25 °C. Growth of all three isolates was inhibited at 35 °C.

Mycelial growth occurred in all three isolates between pH 2.6–8.6, but the maximum extension rates (5.6–6.5 mm day^{-1}) occurred at pH 5.5 (Table 6).

Effect of water potential

Osmotic potential

The fastest hyphal extension rates occurred on PDA osmotically adjusted with NaCl at −1.2 to −2.1 MPa

Table 7. Effect of water potential on radial hyphal extension rate (mm day^{-1}) of three isolates of *P. sydowiana* on PDA at 20 °C

Medium	Isolate	Water potential (−MPa)											
		0.3	0.8	1.2	1.3	2.1	4.8	4.9	6.5	7.8	8.3	9.9	10.5
PDA + NaCl	P5	5.7[1]	5.9	4.9	—[3]	5.6	—	2.1	1.6	—	0.6	0.7	0.0
	P7	4.9	5.4	6.2	—	5.5	—	5.5	3.4	—	2.0	1.4	0.0
	P10	5.3	5.3	6.9	—	5.9	—	4.6	4.1	—	1.8	1.1	0.0
	LSD ($P = 0.05$)[2]	0.43											
PDA + PEG	P5	5.7	5.1	—	5.5	6.4	1.0	—	0.0				
	P7	5.7	4.0	—	3.6	2.9	1.4	—	0.0				
	P10	5.6	3.4	—	3.5	2.4	1.2	—	0.0				
	LSD ($P = 0.05$)[2]	0.62											

[1]Values are means of radial hyphal extension rate (mm day^{-1}) measured between 4 and 6 days on five replicates.
[2]LSD is the least significant difference at a probability of 5% ($P = 0.05$).
[3]Not determined.

for isolates P7 and P10, and at −0.3 to −2.1 MPa for isolate P5 (Table 7). Below these optimal osmotic potentials, extension rates declined markedly with decreasing water potential. Complete inhibition of growth occurred at −10.5 MPa for all three isolates.

Matric potential

The fastest hyphal extension rate on matrically adjusted PDA occurred at −2.1 MPa for isolate P5, and on unamended PDA (−0.3 MPa) for isolates P7 and P10 (Table 7). With further decreases in matric potential, extension rates declined significantly. Growth of all three isolates was completely inhibited at −6.5 MPa.

Discussion

The morphological characteristics of the conidia of the 18 isolates of *Pestalotiopsis*, causing foliar browning, root and stem-base roots of hardy ornamentals, agreed with previous taxonomic descriptions of these characters in *P. sydowiana* (Sutton, 1961, 1980; Hawksworth et al., 1995). Identification of these isolates as *P. sydowiana* was confirmed by experts at CABI Bioscience. More recently, a further 16 *Pestalotiopsis* isolates were obtained by us from *C. vulgaris*, *Erica* sp., *Rhododendron* and conifers in England and Scotland, and the morphological characteristics of most of these also agreed with those of *P. sydowiana*. Our results suggest that *P. sydowiana* could be the predominant species of *Pestalotiopsis* associated with ericaceous plants and conifers in the UK. However, *P. sydowiana* is not the only species which has been isolated elsewhere from hardy ornamentals. For example, in France, Vegh and LeBerre (1992) isolated *P. versicolor* from *Erica* spp. and *P. funerea* from *Cupressus*. Pirone (1978) also reports 12 species of *Pestalotiopsis* causing leaf spots, needle blight, tip blight and grey blight of a range of hardy ornamentals, including *Camellia* (*P. guepini*), *Gardenia* (*P. langloisii*), *Taxus* (*P. funerea*) and *Rhododendron* (*P. macrotricha*).

Three colony types of *P. sydowiana* were distinguished according to top/reverse colour and acervuli production by individual isolates on PDA. Both colour and acervuli production may be sufficiently discriminative characters for identification and classification purposes, provided standard incubation conditions are used. It would be useful in future studies to use RFLP, AFLP or RAPD-PCR analysis to verify our tentative colony morphology-based classification of *P. sydowiana*. Such techniques have already been applied successfully to characterise a range of other fungi (Foster et al., 1993; LoBuglio et al., 1994; Zimand et al., 1994; Matthew et al., 1995; Martin et al., 1998; Hyun and Clark, 1998; Meng et al., 1999).

The pathogenicity tests and host range studies indicated that the isolates of *P. sydowiana* are not host specific and they can infect species of hardy ornamentals other than those from which they were originally isolated. This indicates that *P. sydowiana* has the potential to infect a range of hardy ornamental plants. Isolates were also collected from different locations, confirming the widespread distribution of the pathogen in the UK. In view of the exchange of ericaceous plants and

conifers between the UK and other European countries, it is likely that *P. sydowiana* may have become established throughout most Northern European countries. This merits further investigation.

Pestalotiopsis spp. are primarily thought to act as opportunistic pathogens, and tend to infect plants when they are growing under stress (Coyier and Roane, 1987). Results from the present study have shown that, in order for *P. sydowiana* to infect plants in pathogenicity tests, it is necessary to damage the foliage of unrooted cuttings prior to inoculation with a spore suspension of the pathogen. Previous workers have also found this with other species of *Pestalotiopsis*. For example, White (1930) showed that infection by *P. macrotricha* occurred only when *Rhododendron* leaves were subjected to abrasions, scalding or pinpricks. Similarly, Vegh and LeBerre (1992) reported that *Camellia*, *Rhododendron* and *Thuja* showed symptoms of infection by different species of *Pestalotiopis* after the foliage had been injured by either pesticide damage or sun scald. Consequently, growers may be able to minimise infection by avoiding plant damage and growing plants under extreme stress.

Isolates of *P. sydowiana* grew on all seven media tested. However, SDA, V8, MEA, CDA and PDA were consistently the best for mycelial growth. This confirms the suitability of these media for routine culture of *P. sydowiana* and also suggests that they may be good media on which to study the fungus further.

This investigation has identified the temperature, pH and water potential optima for growth of three isolates of *P. sydowiana*. Although the *in vitro* studies reported here do not directly simulate the conditions of the natural environment, the results provide an insight to the likely behaviour and growth of the pathogen in nature. The isolates were typical mesophiles with optimum hyphal extension rates between 20–25 °C, and little or no growth occurring below 5 °C or above 30 °C. Similar results have been obtained with a number of other species of *Pestalotiopsis* by Vegh and LeBerre (1992). Our results suggest that *P. sydowiana* is well suited as a pathogen of hardy ornamentals grown in temperate climatic conditions such as Europe, and disease development is likely to be inhibited at high and low temperature extremes.

No information relating to pH effects on the growth of *P. sydowiana* has been reported before. In this study, the three isolates of *P. sydowiana* grew over a wide pH range (pH 2.6–8.6), with an optimum at pH 5.5. Since most organic growing media are maintained at pH values in the range 5.0–6.5 (Handreck and Black, 1994), our results suggest that growth of the pathogen is likely to occur in the majority of growing media. Further trials are required to investigate the effect of growing medium pH on the development and survival of *P. sydowiana*.

The water potential of the environment is recognised as an important factor in the ecology of plant pathogenic fungi (Cook and Duniway, 1981; Woods and Duniway, 1986). In soil, pathogens are exposed mainly to matric potential forces, but as they infect plants they become dependent on the water relations of the surrounding plant tissues. This study has revealed for the first time the effects of both osmotic and matric potentials on the growth of *P. sydowiana*. Hyphal extension rates of the three isolates of the pathogen declined with decreasing osmotic potential, with the minimum for growth between −9.9 and −10.5 MPa. These growth responses to osmotic potential are similar to those previously observed for other mitosporic fungi, including several plant pathogens (Cook and Duniway, 1981). The three isolates in this study were also more tolerant of osmotic than matric potential, with no growth occurring at −6.5 MPa on media adjusted with PEG, confirming observations of several plant pathogenic fungi (Adebayo and Harris, 1971; Duniway, 1979; Brownell and Schneider, 1985; Jorge-Silva et al., 1989).

The range of water potentials allowing growth of *P. sydowiana* has important practical implications. Growing media used in ornamental plant production are maintained naturally or artificially at water potentials greater than the permanent wilting point of mesophytic plants (approximately −1.5 MPa) (Slayter, 1967). The growth of *P. sydowiana* at low water potentials may help to explain the greater pathogenicity of the fungus which has often been observed when plants are grown under water stress. It may also explain growers' observations which have revealed a higher incidence and severity of disease on plants subjected to irregular water regimes. The ability of *P. sydowiana* to grow at low water availability must be one of the factors involved in its success as a pathogen of plants grown under water stress.

In conclusion, this study has identified *P. sydowiana* as the main species of *Pestalotiopsis* affecting ericaceous plants and conifers in the UK, as well as some of the morphological and environmental characteristics of selected isolates. Further studies are currently underway to determine the sources of *P. sydowiana* in

nurseries, and to develop effective and reliable control strategies.

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