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Identification and discrimination of *Pseudomonas syringae* isolates from wild cherry in England

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Abstract

A survey of wild cherry (*Prunus avium*) woodland plantations and nurseries was carried out in 2000/01. Trees with symptoms of bacterial canker were found in 20 of the 24 plantations visited and in three of seven nurseries. Fifty-four *Pseudomonas syringae* isolates from wild cherry together with 22 representative isolates from sweet cherry and 13 isolates from other *Prunus* spp., pear and lilac were characterised by physiological, biochemical, serological and pathogenicity tests. Isolates from wild cherry were predominantly *P. syringae* pv. *syringae* (*Pss*), but *P. syringae* pv. *morsprunorum* (*Psm*) races 1 and 2 were also found. Physiological and biochemical tests discriminated *Psm* races 1 and 2 from other *P. syringae* isolates. Agglutination and indirect-enzyme-linked immunosorbent assay tests with three different antisera showed that *Psm* race 1 and race 2 were very uniform and indicated high variability amongst other *P. syringae* isolates. However, pathogenic *Pss* isolates could not be distinguished from non-pathogenic isolates of *P. syringae* on the basis of physiological, biochemical or serological tests. Pathogenicity tests on rooted lilac plants and on micropropagated plantlets of lilac and two wild cherry clones differentiated *Pss* and *Psm* isolates and demonstrated a range of aggressiveness amongst *Pss* isolates. Serological tests could be used as an alternative to the classical physiological and biochemical tests to increase the speed of detection and discrimination of isolates, but pathogenicity tests are still necessary to discriminate the pathogenic *Pss* isolates.

Introduction

Bacterial canker of cherry (*Prunus avium*) caused by *Pseudomonas syringae* is a major limitation in the use of cherry for timber production in farm woodlands in the UK (Nicoll, 1993). The UK Forestry Commission guidelines restrict the proportion of cherry that should be planted in farm woodlands as a direct consequence of bacterial canker (Hibberd, 1988). However, Pryor (1988) found the occurrence of bacterial canker in mature stands of wild cherry to be very limited. Growers are keen to plant higher proportions of cherry due to its short rotation time and the high economic value of the timber. Bacterial canker is also one of the major diseases associated with sweet cherry production and causes significant losses in the hardy nursery stock industry. Copper compounds are commonly used to minimise the spread of canker in sweet cherry orchards, but these compounds have limited efficacy and may have phytotoxic effects. It is not economical or practical to spray farm woodland plantations (Hibberd, 1988). Therefore the only practical approaches to control bacterial canker in farm woodlands are disease avoidance and resistance. Both of these approaches have been limited by a lack of understanding of the pathogens and a lack of reliable and consistent methods for their detection and discrimination.

Previous studies in the UK and other countries have been mainly concerned with sweet cherry. Two pathovars of *P. syringae* cause bacterial canker in

this species: *P. syringae* pv. *morsprunorum* (*Psm*) and *P. syringae* pv. *syringae* (*Pss*). In the UK, bacterial canker of sweet cherry has traditionally been considered to be caused mainly by *Psm* (Wormald, 1937; Crosse, 1955; Garrett et al., 1966; Burkowicz and Rudolph, 1994), whereas in other European countries, South Africa and the USA, the disease of sweet and sour cherries has been attributed to both pathovars of *P. syringae* and to intermediate forms (Crosse and Garrett, 1963; Latorre and Jones, 1979; Roos and Hatting, 1986; Burkowicz and Rudolph, 1994). A pathogenic variant of *Psm* (designated race 2) was identified in East Malling, Kent, UK (Freigoun and Crosse, 1975). More recently *Pss* and/or intermediate forms were also found in sweet cherry in the UK (Garrett and Butler, 1982) and in wild cherry (C.M.E. Garrett and V. Wood, East Malling Research Station, UK, unpublished; Luz, 1997). Isolates of different pathovars and races of *P. syringae* from cherry have been distinguished and characterised by traditional physiological and biochemical tests, hypersensitive reaction of tobacco, pathogenicity tests in fruits and twigs, phage typing and PCR (Garrett et al., 1966; Burkowicz and Rudolph, 1994; Luz, 1997).

The objectives of this work were to identify the pathogens associated with bacterial canker in wild cherry in England, to improve understanding of their taxonomy and variation, and to develop methods for detection and discrimination. This work is a prerequisite for studies on the epidemiology of this disease in farm woodland cherries and for the development of improved methods for disease resistance screening. It may also have wider implications for other cherry producing regions and for bacterial canker in other woody species.

Materials and methods

Survey

Twenty-four woodland plantation sites in eight counties of England (Table 1) and two sweet cherry orchards in a single county (Kent) were visited between September and November 2000. A nursery in Oxfordshire was visited in November 2000 and six nurseries in five other counties of England (Table 1) were visited in April/May 2001. Leaves and branches showing potential symptoms of bacterial canker (cankers and/or leaf spots) were collected. The material was cut from trees using sterile equipment, placed into polythene bags and kept at 4 °C until isolation.

Isolations from diseased material

Small pieces of tissue (~2 mm^2) from leading edges of cankers or from leaf spots were macerated in 300 µl of sterile tap water and allowed to stand for 30 min. The resulting suspensions were plated onto two semi-selective media: modified P3 (mP3) and mineral base sucrose (MS3). mP3 was based on

Table 1. Numbers of English plantations and nurseries at which symptoms of bacterial canker were observed during a survey in 2000–2001

County	Plantations			Nurseries		
	Total	With symptoms[a]	With branch or trunk canker	Total	With symptoms[a]	With branch or trunk canker
Buckinghamshire	2	0	0	—	—	—
Devon	5	5	5	—	—	—
East Sussex	1	1	1	—	—	—
Essex	2	1	0	—	—	—
Gloucestershire	1	1	1	1	0	0
Herefordshire	—	—	—	2	0	0
Kent	6	6	3	1	1	1
Oxfordshire	1	1	1	1	1	0
Shropshire	—	—	—	1	1	1
Suffolk	6	5	1	—	—	—
Worcestershire	—	—	—	1	0	0
Total	24	20	12	7	3	2

[a]Leaf spots and/or cankers.

P3 medium described by Roberts et al. (1996) and consisted of Bacto Pseudomonas Agar F (Difco) supplemented with 10 g l^{-1} glycerol, 0.5 g l^{-1} boric acid, 0.2 g l^{-1} cycloheximide and 0.1 g l^{-1} cephalexin. Boric acid was added to the medium before autoclaving; cycloheximide and cephalexin were added after autoclaving as concentrated stock solutions/suspensions in ethanol. MS3 consisted of 50 g l^{-1} of sucrose, 1 g l^{-1} of $NH_4H_2PO_4$, 0.2 g l^{-1} of KCl, 0.2 g l^{-1} of $MgSO_4.7H_2O$, 0.2 g l^{-1} of chlorothalonil, 0.1 g l^{-1} of bacitracin, 0.05 g l^{-1} of cefuroxime and 12 g l^{-1} of agar (Oxoid no. 3). The pH of MS3 was adjusted to 7.0–7.2 before adding the agar; the antibiotics and the fungicide were added after autoclaving as concentrated stock solutions/suspensions in ethanol for chlorothalonil and cefuroxime and in 50% ethanol for bacitracin. Cream/yellow, blue fluorescent and non-fluorescent colonies on mP3 and levan colonies on MS3 were considered as suspect *P. syringae* and tested by *Staphylococcus aureus* slide agglutination (Lyons and Taylor, 1990) with three *P. syringae* antisera (described below). Colonies that tested positive in at least one agglutination test were transferred to King's medium B (KB) (King et al., 1954) and preserved at −76 °C (Feltham et al., 1978) in liquid medium containing 8 g l^{-1} of nutrient broth (Difco) and 150 ml l^{-1} of glycerol.

Bacterial isolates

Thirty-five isolates obtained in this study from wild and sweet cherry plus 41 isolates obtained by other researchers, mainly from the UK, were characterised (Table 2). Thirteen representative isolates of *P. syringae* from other hosts including plum (*Prunus domestica*), cherry laurel (*Prunus laurocerasus*), peach (*Prunus persica*), myrobalan (*Prunus cerasifera*), pear (*Pyrus communis*) and lilac (*Syringa vulgaris*) were also included. All isolates were recovered from −76 °C and grown on KB at 25 °C.

Physiological and biochemical tests

Cultures were incubated at 25 °C unless otherwise stated. Isolates were tested for oxidase activity (Kovacs, 1956). Fluorescence on KB medium was checked under UV light after 1–3 d, and production of brown pigment on this medium was recorded after 4 d. Levan production on nutrient agar (Difco) with 50 g l^{-1} sucrose was recorded after 1 and 2 d. The colour of growth in nutrient broth (Difco) with 50 g l^{-1} sucrose was examined from 2 to 6 d (Crosse and Garrett, 1963). The GATTa tests of Latorre and Jones (1979) were performed with some modifications. Gelatin liquefaction (G) was tested in tubes containing 120 g l^{-1} gelatin stabbed with bacterial growth; after incubation at 20 °C for 14 d the tubes were transferred to 4 °C for 30 min before the results were scored. β-glucosidase activity (A) was tested in tubes containing 1 g l^{-1} aesculin liquid medium (Sneath, 1956); the cultures were incubated at 20 °C and examined from 1 to 4 d. Tyrosinase activity (T) was tested on agar containing 1 g l^{-1} L-tyrosine (Lelliott et al., 1966); results were recorded after 2–4 d. Utilisation of tartrate as a sole carbon source (Ta) was tested on the basal agar of Ayers et al. (1919) with 2 g l^{-1} sodium tartrate; results were recorded after 7 and 14 d.

Serological tests

Isolates were tested by *S. aureus* slide agglutination (Lyons and Taylor, 1990) with the antisera 00/8/3, 00/9/3 and 105D. These antisera were produced from live whole-cell antigens (Lyons and Taylor, 1990). Antiserum 00/8/3 was produced against *Psm* isolate HRI 5270 and 00/9/3 was produced against *Pss* isolate HRI 5275; both isolates were from wild cherry. Antiserum 105D was produced previously against a *P. syringae* isolate (HRI 245A) from pea (*Pisum sativum*). The optimum dilution of antisera for conjugation was determined by titration. The following quantities were used: antiserum diluted in phosphate-buffered saline with 0.2 g l^{-1} sodium azide (PBSA) 800 µl, *S. aureus* working reagent 160 µl and filtered, saturated alcoholic basic fuchsin 20 µl. Antiserum 00/8/3 was used at a dilution of 1 : 40 in PBSA and 00/9/3 was used at 1 : 80; 105D, initially mixed with glycerol (1 : 1), was further diluted 1 : 10 in PBSA. After preparation, the conjugate was stored at 4 °C overnight prior to use. Bacterial growth from a plate was mixed into 10 µl aliquots of conjugate on a multitest microscope slide using a sterile toothpick. The results were recorded on a five point scale: 0, no agglutination; 1, weak non-specific reaction; 2, slow (more than 1 min) positive reaction; 3, positive agglutination in 30 s to 1 min; 4, positive agglutination in less than 30 s.

Isolates were tested by indirect-enzyme-linked immunosorbent assay (ELISA) with the antisera 00/8/3, 00/9/3 and 105D. Forty-eight hour bacterial growth from plates of KB was harvested and diluted in PBSA to obtain bacterial suspensions with an

Table 2. Sources and origin of 54 *P. syringae* isolates from wild cherry, 22 isolates from sweet cherry and 13 isolates from other hosts, grouped according to the results of the physiological and biochemical tests as well as pathogenicity tests on rooted plants and micropropagated plantlets of lilac

HRI–W isolate number (original designation)	Fluorescence on KB (browning)[a]	Source	Host	Material	Country/County (source)	Year of isolation
$G^+A^+T^-Ta^-$, yellow growth in NSB (P. syringae *pv.* syringae *group*)						
Strongly pathogenic on rooted and micropropagated lilac						
5267 (AV114)	NF	C.M.E. Garrett	Wild cherry	Leaf wash	UK/Buckinghamshire	1990
5272 (AV180)	NF	C.M.E. Garrett	Wild cherry	Leaf wash	UK/Oxfordshire	1990
5275 (AV200)	BF	C.M.E. Garrett	Wild cherry	Canker	UK/Kent	1990
5276 (AV208), 5277 (AV210)	BF	C.M.E. Garrett	Wild cherry	Leaf wash	UK/Kent	1990
5835 (R8)	BF	J.P. Luz	Wild cherry	Branch canker	UK/Berkshire	1994
5841 (11L-F3)	WBF	J.P. Luz	Wild cherry	Branch canker	UK/West Sussex	1995
7924	BF	This study	Wild cherry	Canker	UK/Suffolk	2000
7928A	BF	This study	Wild cherry Accession no. 2515	Leaf spots	UK/Kent	2000
7929B	BF	This study	Wild cherry selection FD1-57-4-68	Leaf spots	UK/Kent	2000
7972	BF	This study	Wild cherry	Leaf spots	UK/Essex	2000
8094A, 8094B, 8094C	BF	This study	Wild cherry	Canker	UK/Kent (nursery)	2001
5262 (C709)	BF	C.M.E. Garrett	Sweet cherry cv. Van	Leaf spots	UK	
5355 (S113)	BF		Sweet cherry cv. Napoleon		France	1978
5356A (S151A)	BF	C.M.E. Garrett	Sweet cherry		UK	
5357 (S157)	BF	C.M.E. Garrett	Sweet cherry cv. Van	Leaf spot	UK/Kent	1979
7874	BF	C. Lewis	Sweet cherry cv. Valera	Canker	UK/Kent	2000
7926A	BF	This study	Sweet cherry cv. Gaucher	Leaf spots	UK/Kent	2000
7933, 7973A	BF	This study	Sweet cherry cv. Gaucher	Canker	UK/Kent	2000
SC073B	BF	S.J. Roberts	Cherry laurel	Leaf spot	UK	1982
801 (NCPPB281)	BF	K.A. Sabet	Lilac		UK	1950
2070	BF	S.J. Roberts	Lilac			1988
5340 (S4)	BF	C.M.E. Garrett	Pear cv. Conference	Shoot	UK/Sussex	1959
7872	BF	C. Lewis	Plum cv. Opal	Canker	UK/Kent	2000
7873	BF	C. Lewis	Plum cv. Excalibur	Canker	UK/Kent	2000
Weakly pathogenic on rooted lilac, strongly pathogenic on micropropagated lilac						
5264 (AV78)	BF	C.M.E. Garrett	Wild cherry	Leaf wash	UK/Kent	1990
7921	BF	This study	Wild cherry	Leaf spots	UK/Suffolk	2000
7963	BF	This study	Wild cherry cv. F12/1	Leaf spots	UK/Oxfordshire (nursery)	2000
7964, 7965A	BF	This study	Wild cherry selection FD1-57-4/122	Leaf spots	UK/Oxfordshire (nursery)	2000
Very weak/not pathogenic on rooted lilac, pathogenic on micropropagated lilac						
5265 (AV81)	BF	C.M.E. Garrett	Wild cherry	Leaf wash	UK/Kent	1990
7919A	BF	This study	Wild cherry	Leaf spots	UK/Suffolk	2000

Table 2. (*Continued*)

HRI–W isolate number (original designation)	Fluorescence on KB (browning)[a]	Source	Host	Material	Country/County (source)	Year of isolation
7922A	BF	This study	Wild cherry	Leaf spots	UK/Suffolk	2000
7955C	WBF	This study	Wild cherry	Leaf spots	UK/Devon	2000
7956	BF	This study	Wild cherry cv. F12/1	Leaf spots	UK/Oxfordshire (nursery)	2000
7962	BF	This study	Wild cherry cv. F12/1	Leaf spots	UK/Oxfordshire (nursery)	2000
7971A	BF	This study	Wild cherry	Leaf spots	UK/Essex	2000
Very weak/not pathogenic on rooted and micropropagated lilac						
5828 (1AH-B)	BF	J.P. Luz	Wild cherry	Trunk canker	UK/Hampshire	1995
5837 (11L-E1)	BF	J.P. Luz	Wild cherry	Branch canker	UK/West Sussex	1995
7920B	BF	This study	Wild cherry	Leaf spots	UK/Suffolk	2000
7927A	BF	This study	Wild cherry	Leaf spots	UK/East Sussex	2000
7928C	BF	This study	Wild cherry Accession no. 2515	Leaf spots	UK/Kent	2000
7929A	NF	This study	Wild cherry selection FD1-57-4-68	Leaf spots	UK/Kent	2000
7929D	WBF	This study	Wild cherry selection FD1-57-4-68	Leaf spots	UK/Kent	2000
7932A	BF	This study	Wild cherry (rootstock)	Leaf spots	UK/Kent	2000
7949	BF	This study	Wild cherry	Leaf spots	UK/Oxfordshire	2000
7959, 7961	BF	This study	Wild cherry cv. F12/1	Leaf spots	UK/Oxfordshire (nursery)	2000
7969, 7970A	BF	This study	Wild cherry	Leaf spots	UK/Essex	2000
2942	BF	R. Samson	Sweet cherry		New Zealand	
$G^-A^-T^+Ta^+$, *white growth on NSB* (P. syringae *pv.* morsprunorum *race 1 group*), *not pathogenic on lilac*						
5266 (AV93)	NF (B)	C.M.E. Garrett	Wild cherry	Leaf wash	UK/Surrey	1990
5269 (AV122), 5270 (AV125A)	NF (B)	C.M.E. Garrett	Wild cherry	Branch canker	UK/Buckinghamshire	1990
5833 (NA7)	NF (B)	J.P. Luz	Wild cherry	Trunk canker	UK/Oxfordshire (nursery)	1994
8133	NF (B)	This study	Wild cherry	Leaf spots	UK/Shropshire (nursery)	2001
798 (NCPPB1781)	NF (B)	G.L. Ercolani	Sweet cherry		Italy	1965
2206 (NCPPB1463)	NF (B)	C.M.E. Garrett	Sweet cherry		UK	1961
5238 (C5; NCPPB1459)	WBF (B)	J.E. Crosse	Sweet cherry cv. Napoleon	Leaf wash	UK/Kent	1957
5239 (C9A, NCPPB1460)	NF	J.E. Crosse	Sweet cherry	Leaf wash	UK/Kent	1957
5243 (C22, NCPPB1462)	NF (B)	J.E. Crosse	Sweet cherry cv. Napoleon	Leaf wash	UK/Kent	1960
5244 (C28A)	NF (B)	J.E. Crosse	Sweet cherry	Seedling canker	UK	1960
5259 (C520)	WBF	C.M.E. Garrett	Sweet cherry			
797 (NCPPB1095)	BF (B)	E. Billing	Plum		UK	1960
2928	BF (B)	T. Burki	Plum		Switzerland	1965
5281 (D17)	NF (B)	C.M.E. Garrett	Plum cv. Victoria	Leaf spot	UK/Kent	1961
5299 (JP140)	WBF (B)	C.M.E. Garrett	Plum		UK	
5300 (JP168)	NF (B)	C.M.E. Garrett	Plum		UK	

Table 2. (*Continued*)

HRI–W isolate number (original designation)	Fluorescence on KB (browning)[a]	Source	Host	Material	Country/County (source)	Year of isolation
$G^+A^-T^-Ta^-$, white growth on NSB (P. syringae *pv.* morsprunorum *race 2 group*), *not pathogenic on lilac*						
5271 (AV156)	NF	C.M.E. Garrett	Wild cherry	Leaf wash	UK/Gloucestershire	1990
7958A	BF	This study	Wild cherry cv. F12/1	Leaf spots	UK/Oxfordshire (nursery)	2000
5250 (C192)	NF	C.M.E. Garrett	Sweet cherry cv. Napoleon	Leaf spot	UK/Kent	1971
5252 (C301)	NF	C.M.E. Garrett	Sweet cherry cv. Roundel	Leaf wash	UK/Kent	1971
5253 (C309)	NF	C.M.E. Garrett	Sweet cherry cv. Napoleon		UK/Kent	1971
5255 (C331 RA)	NF	J.P. Prunier	Sweet cherry cv. Napoleon		UK?	
5260 (C613)	NF	C.M.E. Garrett	Sweet cherry cv. Roundel		UK	
5261 (C651)	NF	C.M.E. Garrett	Sweet cherry cv. Roundel	Canker	UK	
$G^+A^+T^-Ta^-$, white or intermediate colour growth in NSB[b] (P. syringae *intermediate group*), *not pathogenic on lilac*						
SC214, SC217	BF	S.J. Roberts	Wild cherry	Shot hole	UK/Oxfordshire	1983
5268 (AV119)	BF	C.M.E. Garrett	Wild cherry	Leaf wash	UK/Buckinghamshire	1990
5831 (M1A)	BF	J.P. Luz	Wild cherry	Branch canker	UK/Oxfordshire	1994
5836 (11L-B)	BF	J.P. Luz	Wild cherry	Branch canker	UK/West Sussex	1995
5845 (13/14 EF-C)	BF	J.P. Luz	Wild cherry	Leaf wash	UK/Surrey	1995
7967A, 7968A	BF	This study	Wild cherry	Leaf spots	UK/Essex	2000
Others						
$G^+A^-T^+Ta^-$, white growth in NSB, not pathogenic on lilac						
5400 (EP20, NCPPB2075)	NF	C.M.E. Garrett	Myrobalan	Shoot wilt	UK	1966
$G^-A^-T^-Ta^-$, intermediate colour growth in NSB, not pathogenic on lilac						
5402 (E116, NCPPB2762)	NF	J. Luisetti	Peach		France	1974

[a]NF, non-fluorescent; BF, blue fluorescent; WBF, weakly blue fluorescent; (B) browning of the medium.
[b]Isolate 5831 produced an intermediate colour (yellow/white) growth in NSB; other isolates produced white growth.

optical density of 0.4. ELISA tests were performed on different occasions with three different sets of isolates, but always including a *Psm* race 1 isolate (HRI 5270), a *Psm* race 2 isolate (HRI 5260) and a *Pss* isolate (HRI 5275) for comparison. Each plate also included a reagent positive control (*P. syringae* pv. *avellanae* isolate HRI 6015, and its homologous antiserum 96/1/3) and a blank (no antigen). ELISA plates were coated with antigen by incubating duplicate 100 µl aliquots of bacterial suspensions at 4 °C overnight. Between steps, plates were washed with a solution of 1.5 mM KH_2PO_4, 8.5 mM Na_2HPO_4, 0.14 M NaCl, 2.7 mM KCl, pH 7.2, containing 1 mg l^{-1} basic fuchsin and 1 mg l^{-1} thimerosal (PBST) in a Swatwash plate washer (Luminar Technology Ltd., Southampton, UK) programmed to perform four wash cycles with a 60 s soak time. Plates were blocked with PBST containing 50 g l^{-1} bovine serum albumin suspension (200 µl per well) for 1 h at room temperature. Plates were then incubated with primary antibody (100 µl per well) for 45 min in a shaker at 37 °C. The titre of each antiserum was determined previously by titration with the homologous isolate. Antisera 00/8/3 and 00/9/3 were used at 1 : 3200, 105D was used at 1 : 800 and the reagent control 96/1/3 was used at 1 : 1500. Plates were incubated with an antirabbit IgG antibody conjugated to alkaline phosphatase (Sigma) (100 µl per well) at 37 °C for 45 min in a shaker. Absorbances were recorded after incubation in the dark for 30 min with 1 mg ml^{-1} of substrate p-nitrophenyl phosphate in 1 M diethanolamine, pH 9.8 (100 µl per well), on a Anthos Labtech HTII plate reader at 405 nm

with a reference at 620 nm and were corrected for blanks.

Pathogenicity tests on micropropagated plantlets

Isolates were tested for pathogenicity on micropropagated plantlets of two wild cherry clones (cv. Charger and accession number 1912) and lilac cv. Sensation using a method based on that of Scheck et al. (1997). Plant cultures were maintained in 71 mm diameter transparent plastic pots (C71-141, Insulpak, Huntingdon) with periodic sub-culturing. Cherry cultures were maintained on a shoot culture medium based on that of Hammatt and Grant (1993) consisting of Murashige and Skoog (1962) salts supplemented with 87.6 mM sucrose, 2.2 μM benzyladenine, 0.49 μM indole-3-butyric acid, 1.28 mM phloroglucinol, 6 g l^{-1} of agar and adjusted to a pH of 5.65. Lilac cultures were maintained on lilac tissue culture medium based on that of Scheck et al. (1997) consisting of Murashige and Skoog (1962) salts supplemented with 87.6 mM sucrose, 8.9 μM benzyladenine, 6 g l^{-1} of agar and adjusted to a pH of 5.2. Bacterial isolates were grown on KB at 25 °C for 48 h before inoculation. Growth was scraped from the plates and suspended in sterile tap water to produce a suspension containing 10^8–10^9 cfu ml^{-1}. Two- to three-week-old cultures of the two wild cherry clones and lilac were inoculated by dipping the plantlets into the bacterial suspension. Each isolate was inoculated into three plants of each clone in a pot. The plants were maintained at 25 ± 2 °C with a 16 h photoperiod. Results were recorded weekly for 4 weeks. The severity of symptoms was assessed on a six point scale: 0, no symptoms; 1, small necrotic or chlorotic areas in one or two leaves; 3, less than half of the plantlet with necrotic or chlorotic lesions; 5, half of the plantlet with necrotic or chlorotic lesions; 7, more than half of the plantlet with necrotic stem and leaves; 9, dead plantlet. The presence of leaf spots was also recorded (number of leaf spots counted). Plantlets with a score of 0, 1, 3 were considered resistant; plantlets with a score of 5 were considered susceptible and plantlets with 7 or 9 were considered very susceptible.

Pathogenicity tests on rooted lilac plants

Isolates were tested for pathogenicity on rooted plants of lilac cv. Sensation using the method of Young (1991) with some modifications. Plants were maintained in 2 l plastic pots in a glasshouse with a minimum temperature of 22/17 °C (day/night) and venting at 24/19 °C (day/night). Plants were pruned to stimulate the production of new shoots. Young (∼3-week-old) shoots were inoculated by stabbing the stem near the tip, the leaves at six points and the petioles of several opposing leaves with an entomological pin charged with bacterial growth. Symptoms were recorded weekly for 4 weeks. Isolates that caused progressing dark lesions surrounding the inoculation point at the petioles and chlorotic and/or necrotic lesions on the leaf laminae were considered pathogenic.

Results

Survey and isolations

Trees with bacterial disease symptoms (leaf spots and/or cankers) were found in most (20 out of 24) woodland plantation sites visited, with trunk and/or branch cankers in half of them (Table 1). However, although present, bacterial canker was not a major problem in most plantations and just a small proportion of the trees showed symptoms. One exception was a young plantation (trees less than 10-years-old) in Oxfordshire where nearly all trees were severely affected. In many plantations, the trees were morphologically heterogeneous, varying from wild to sweet cherry types. In contrast to wild cherry, sweet cherry types are characterised by poor apical growth, heavy branching, open habit, numerous buds forming fruiting spurs and large fruits. In some cases the most severely affected trees were more similar to the sweet cherry type. The disease generally occurred with lower frequency or was less severe in older trees. Potential *P. syringae* isolates were obtained from ∼33% of the samples collected (18% of isolation attempts). *P. syringae* isolates were also obtained from samples of sweet cherry cv. Gaucher collected in two sweet cherry orchards in Kent. The isolates obtained in this study are listed in Table 2.

Trees in three of the seven nurseries visited showed symptoms of bacterial canker (Table 1). Trees at most of the nurseries were grown from seed obtained in the UK and/or eastern Europe (e.g. Hungary, Poland). Trees at one nursery in Oxfordshire had been produced by micropropagation. Isolates were obtained from leaf spots at two nurseries (in Oxfordshire and in Shropshire) and from cankers in surplus stock (un-sold older trees) at a nursery in Kent. These isolates are included in Table 2.

Physiological and biochemical tests

The isolates from wild cherry, sweet cherry, plum and other hosts characterised in this study were oxidase negative and levan positive on nutrient sucrose agar and gave variable reactions in other tests. The results are summarised in Table 3. Most of the isolates from wild cherry produced yellow growth in NSB, were positive for gelatin liquefaction and aesculin hydrolysis, but were negative for tyrosinase activity and tartrate utilisation ($G^+A^+T^-Ta^-$); these isolates were considered to be *Pss* according to the definitions of Garrett et al. (1966), Freigoun and Crosse (1975) and Latorre and Jones (1979). Five isolates from wild cherry were non-fluorescent, produced a brown pigment on KB, white growth in NSB and were $G^-A^-T^+Ta^+$; these isolates were considered to be *Psm* race 1 according to the previous definitions. Two wild cherry isolates produced white growth in NSB and were $G^+A^-T^-Ta^-$; these isolates were considered to be *Psm* race 2. The remaining eight wild cherry isolates produced either white or an intermediate, yellow/white, coloured growth in NSB and were $G^+A^+T^-Ta^-$; these isolates were considered to be intermediate forms.

The isolates from sweet cherry were divided into three groups, *Pss*, *Psm* race 1 and *Psm* race 2, and the isolates from plum were either included in the *Pss* or in the *Psm* race 1 group. Isolates from lilac, cherry laurel and pear were included in the *Pss* group. The isolate from myrobalan produced white growth in NSB and was $G^+A^-T^+Ta^-$ and the isolate from peach (*P. syringae* pv. *persicae*) produced intermediate coloured growth in NSB and was $G^-A^-T^-Ta^-$; these two isolates could not be included in any of the previous groups.

Serological tests

The results of agglutination tests and indirect-ELISA with the antisera 00/8/3 (raised against a wild cherry *Psm* race 1 isolate), 00/9/3 (raised against a wild cherry *Pss* isolate) and 105D (raised against a *P. syringae* isolate from pea) are summarised in Table 4. Forty-nine of 54 isolates from the *Pss* group ($G^+A^+T^-Ta^-$, yellow growth in NSB) agglutinated with both 00/9/3 and 105D antisera; the majority of these isolates did not react with 00/8/3. The isolates from the *Psm* race 1 group ($G^-A^-T^+Ta^+$, white growth in NSB) agglutinated with 00/8/3, but not with 00/9/3 and 105D, with the exception of two isolates (HRI 2928 and 5300) from plum. The *Psm* race 2 ($G^+A^-T^-Ta^-$, white growth in NSB) and intermediate group isolates ($G^+A^+T^-Ta^-$, white or yellow/white growth in NSB) agglutinated with both 00/8/3 and 00/9/3, with the exception of the isolate HRI 5271 from wild cherry. Three isolates (HRI 5271, HRI 5400 from myrobalan and HRI 5402 from peach) did not agglutinate with any of the antisera tested.

The results obtained with the reagent positive control and the three control isolates were consistent between indirect-ELISA plates. The reagent positive control (*P. syringae* pv. *avellanae* isolate HRI 6015 with its homologous antiserum 96/1/3) always gave an absorbance value greater than 1.3. In all three plates tested with the antisera 00/8/3, the *Psm* isolates HRI 5260 and HRI 5270 gave a strong positive reaction (absorbance $\geq$1.0), but the *Pss* isolate HRI 5275 did

Table 3. Summary of physiological and biochemical tests on the 89 isolates examined in this study

Number of isolates	Hosts of origin (number of isolates)	Fluorescence on KB	Colour of growth in NSB	Results of GATTa[a] tests	Group
54	Wild cherry (39), sweet cherry (9), plum (2), lilac (2), cherry laurel (1), pear (1)	Variable	Yellow	$G^+A^+T^-Ta^-$	*Pss*
17	Wild cherry (5), sweet cherry (7), plum (5)	Non-fluorescent or weakly blue fluorescent	White	$G^-A^-T^+Ta^+$	*Psm* race 1
8	Wild cherry (2), sweet cherry (6)	Variable	White	$G^+A^-T^-Ta^-$	*Psm* race 2
8	Wild cherry (8)	Blue	White or yellow/white	$G^+A^+T^-Ta^-$	Intermediate
1	Myrobalan	Non-fluorescent	White	$G^+A^-T^+Ta^-$	Other pathovar
1	Peach	Non-fluorescent	Yellow	$G^-A^-T^-Ta^-$	Other pathovar

[a]Results of four tests: G, gelatin liquefaction; A, aesculin hydrolysis; T, tyrosinase activity; Ta, tartrate utilisation.

Table 4. Summary of agglutination and ELISA tests on 89 isolates

Number of isolates	Agglutination with antiserum (score range[a])			ELISA with antiserum (absorbance range[a])			Hosts[b] (number of isolates)
	00/8/3	00/9/3	105D	00/8/3	00/9/3	105D	
Pss *group ($G^+A^+T^-Ta^-$, yellow growth in NSB)*							
34	0–1	3–4	3–4	0.0–0.3	1.0–2.0	1.0–2.2	w (10), s (1), p (2), cl (1)
				0.0–0.1	1.7–2.1	0.7–0.9	w (2), s (1)
				0.1–0.3	0.2–0.8	1.0–2.4	w (8), s (3), l (2)
				0.1–0.2	0.4–0.7	0.5–0.8	w (4)
15	3–4	3–4	3–4	1.1–1.8	0.5–0.9	1.2–1.8	w (4), s (1)
				1.1-1.5	0.3–0.6	0.4–0.7	w (2), pr (1)
				1.7–1.8	1.0–1.1	1.9–2.1	w (2)
				0.6–0.8	0.6–0.7	1.2–1.6	w (2)
				0.3–0.9	0.3–0.5	0.5–0.8	w (2), s (1)
4	0–1	3–4	1–2	0.1	1.8	0.6	s (1)
				0.1	0.6	1.3	w (1)
				0.1–0.7	0.3–0.4	0.2–0.6	w (1), s(1)
1	3	0	3	0.2	0.4	0.3	w (1)
Psm *race 1 group ($G^-A^-T^+Ta^+$, white growth in NSB)*							
15	3-4	0–1	0–1	1.2–2.8	0.2–0.6	0.0–0.4	w (5), p (3), s (7)
2	4	3–4	0–1	0.4–0.6	0.7–1.1	0.1–0.3	p (2)
Psm *race 2 group ($G^+A^-T^-Ta^-$, white growth in NSB)*							
7	4	3–4	0–1	1.5–2.0	0.4	0.1–0.7	w (1), s (6)
1	0	0	1	0.3	0.3	0.2	w (1)
Intermediate group ($G^+A^+T^-Ta^-$, white or yellow/white growth in NSB)							
8	3–4	3–4	0–1	1.2–1.9	0.2–0.5	0.1–0.2	w (8)
Others (isolates 5400, 5402)							
2	0	0	1–2	0.2–0.3	0.3	0.2–1.0	my (1), ph (1)

[a]Underlined values were considered positive.
[b]Hosts: w, wild cherry; s, sweet cherry; p, plum; cl, cherry laurel; l, lilac, pr, pear; my, myrobalan; ph, peach.

not (absorbance <0.2). In the plates tested with 00/9/3 and 105D, the *Psm* isolates HRI 5260 and HRI 5270 did not react well (absorbance ≤0.5), but the isolate HRI 5275 gave a strong positive reaction (absorbance ≥1.2). Based on these results, isolates that produced absorbances greater or equal to 1.0 were considered strongly positive.

Isolates from the *Pss* group gave variable reactions in indirect-ELISA (Table 4). Forty-one of these isolates were positive (absorbance ≥1.0) with the antisera 00/9/3 and/or 105D; 44 isolates were negative (absorbance <1.0) with 00/8/3. Isolates from the *Psm* race 1, *Psm* race 2 and *Ps* intermediate groups were positive (absorbance ≥1.2) with 00/8/3 and gave weak reactions (absorbance <0.8) with 00/9/3 and 105D with three exceptions (two isolates from plum, 2928, 5300, and one isolate from wild cherry, 5271). The isolates from myrobalan and peach did not react well with the antisera against cherry isolates (00/8/3 and 00/9/3).

Pathogenicity tests on micropropagated plantlets and on rooted lilac plants

Results of pathogenicity tests on micropropagated plantlets of lilac (cv. Sensation) and wild cherry (cv. Charger and accession number 1912) and rooted plants of lilac (cv. Sensation) are summarised in Table 5. The isolates from the *Pss* group were highly variable: 28 isolates were strongly pathogenic on micropropagated plantlets of lilac and cherry (Figure 1) and on rooted lilac. In rooted plants, the symptoms were easier to interpret in petioles than on the leaf laminae and shoot tips. Five isolates were strongly pathogenic on lilac, but were less aggressive on the cherry plantlets and on rooted lilac plants and seven isolates showed intermediate pathogenicity on both lilac and cherry plantlets and did not produce symptoms on rooted lilac. Fourteen isolates were very weak or non-pathogenic on lilac and cherry plantlets and on rooted lilac. In total,

Table 5. Summary of pathogenicity tests on rooted lilac cv. Sensation, on micropropagated plantlets of lilac cv. Sensation and of wild cherry cv. Charger and accession 1912 with 89 isolates

Number of isolates	Hosts[a] (number of isolates)	Pathogenicity on mature lilac plants	Pathogenicity on micropropagated plantlets			
			Range of means[b] on			Leaf spots on cherry
			Lilac	Charger	1912	
Pss *group ($G^+A^+T^-Ta^-$, yellow growth in NSB)*						
28	w (14), s (8), cl (1), p (2), l (2), pr (1)	+	7.0–9.0	6.3–9.0	6.0–9.0	–
5	w (5)	(+)	6.7–9.0	1.7–6.0	1.0–5.0	–
7	w (7)	–	3.7–7.0	1.3–6.0	2.0–7.3	–
14	w (13), s (1)	–	0.0–3.0	0.2–1.7	0.7–2.8	–
Psm *race 1 group ($G^-A^-T^+Ta^+$, white growth in NSB)*						
10	w (4), s (5), p (1)	–	0.0–3.0	4.3–7.0	1.7–8.3	+[c]
7	w (1), s (2), p (4)	–	0.0–1.3	0.0–3.0	0.0–3.0	+[d]
Psm *race 2 group ($G^+A^-T^-Ta^-$, white growth in NSB)*						
8	w (2), s (6)	–	0.0–2.3	1.0–6.3	0.7–3.7	+[e]
Intermediate group ($G^+A^+T^-Ta^-$, white or yellow/white growth in NSB)						
8	w (8)	–	0.2–1.7	0.7–3.2	0.7–3.7	+[f]
Others						
2	my, (1), ph (1)	–	1.7	2.3–3.0	0.3–1.3	–

[a]Hosts: w, wild cherry; s, sweet cherry; p, plum; cl, cherry laurel; l, lilac, pr, pear; my, myrobalan; ph, peach.
[b]Underlined values were considered positive (pathogenic).
[c]Nine of these isolates (*Psm* race 1), [d]Three of these isolates (*Psm* race 1), [e]Six of these isolates (*Psm* race 2), [f]Four of these isolates (intermediate) produced leaf spots on plantlets of Charger and/or 1912.

21 isolates from the *Pss* group did not cause symptoms on the rooted plants, but seven of these isolates were still pathogenic (mean scores greater than 3) on lilac micropropagated plantlets.

Isolates from all other groups were not pathogenic on micropropagated lilac plantlets (mean scores of 3 or less) and rooted lilac plants. Most isolates from the *Psm* race 1 group were pathogenic on cherry plantlets and produced leaf spots in at least one of the clones (Figure 1). Seven isolates (from wild cherry, sweet cherry and plum) were weak on the wild cherry clones although leaf spots were observed in plants inoculated with some of these isolates. The isolates from the *Psm* race 2 and intermediate groups were generally weak in the wild cherry plantlets, but ten of these isolates produced leaf spots. The isolates from *P. cerasifera* and *P. persica* were not pathogenic on either lilac or cherry.

Discussion

Bacterial canker was found in wild cherry in most of the counties of England which were surveyed. Previous surveys of wild cherry in England in 1989 and 1990 (C.M.E. Garrett and V. Wood, East Malling Research Station, UK, unpublished) showed that the disease was present in Buckinghamshire, Essex, Gloucestershire, Kent, Oxfordshire and Worcestershire and in 1994 and 1995 in Berkshire, Hampshire, Oxfordshire, Surrey and West Sussex (Luz, 1997). Thus bacterial canker is present throughout southern England and should be considered a permanent threat to wild cherry production in this country.

In most of the plantations visited, only a small proportion of the trees showed symptoms of bacterial canker. This may be due to variable levels of resistance: most wild cherry trees are raised from seed and are likely to be genetically very variable. The disease seems to occur at a higher frequency or to be more severe in young trees. This may be because older trees might be more resistant to bacterial canker and/or these trees might be the survivors from early attacks of the disease. As wild cherry is generally planted as a proportion of a mixed plantation, this might also limit the spread and build up of inoculum.

Symptoms of bacterial canker were found in three nurseries indicating that they can be a source of the disease. In the young plantation in Oxfordshire, where the cherry trees were all severely affected and most were not expected to survive, the disease probably was initially spread in the nursery.

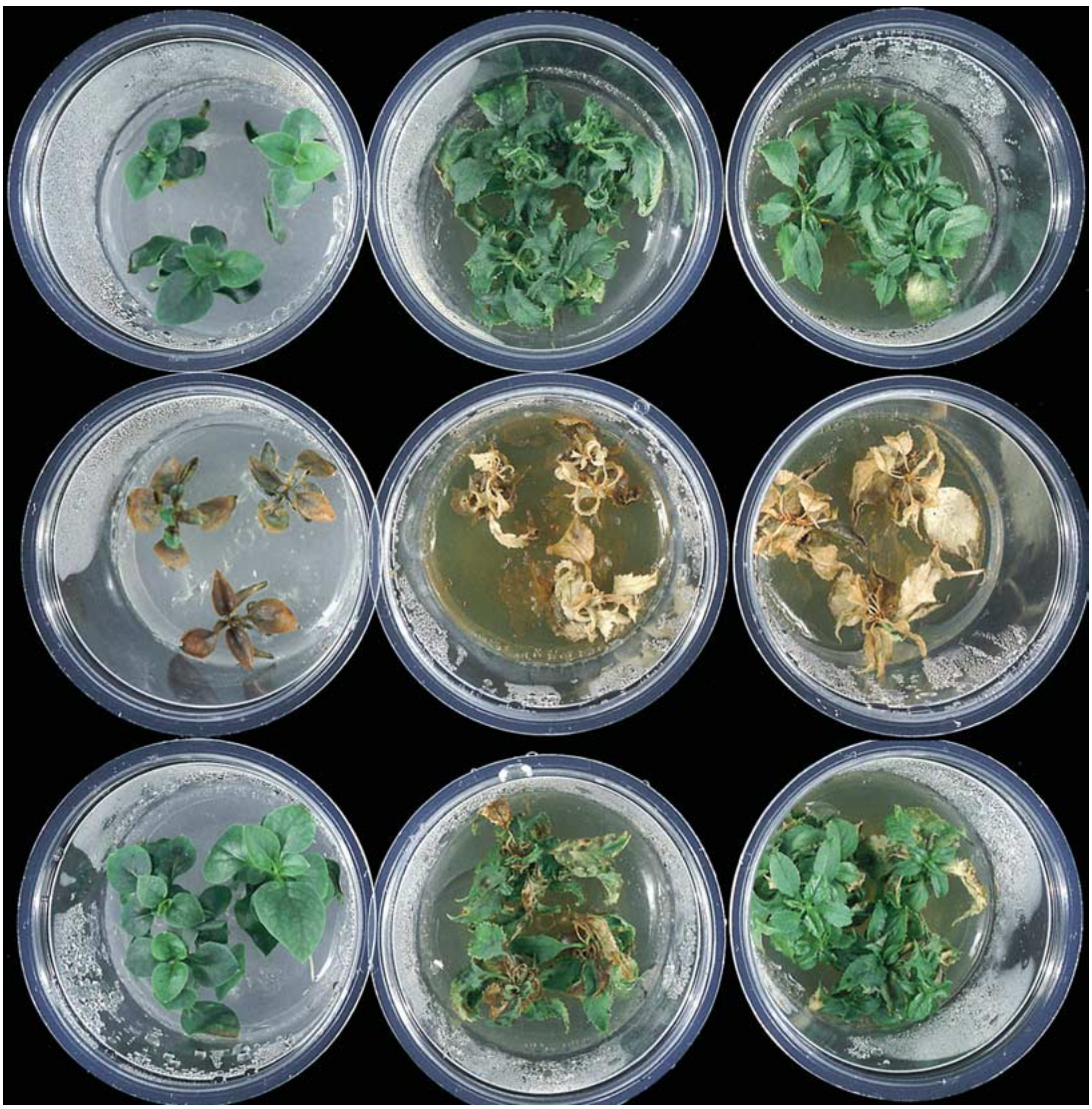

Figure 1. Micropropagated plantlets of lilac cv. Sensation (left), and wild cherry cv. Charger (middle) and accession 1912 (right), inoculated with sterile tap water (top row), *Pss* (HRI 5841) (middle row) and *Psm* race 1 (HRI 5266) (bottom row).

Isolation of *P. syringae* from plant material showing bacterial canker symptoms was generally difficult (isolation success of 18%). The two semi-selective media used in this study were developed for isolation of *P. syringae* isolates from cherry. A larger number of isolates were obtained from leaf spots than from branch cankers. This was probably related to the time of the year (September–November) when plantations were visited. Whilst cankers have a peak of development in spring and most bacteria die or become inactive during the summer, the summer phase of the disease on green organs begins in Spring and secondary infections occur throughout the summer on leaves of extension shoots (Crosse, 1966).

Differentiation of *P. syringae* isolates into pathovars *syringae* and *morsprunorum* on the basis of biochemical and physiological tests as suggested by previous authors (Crosse and Garrett, 1963; Garrett et al., 1966; Freigoun and Crosse, 1975; Latorre and Jones, 1979; Roos and Hatting, 1986; Burkowicz and Rudolph, 1994) was generally supported by our results. The GATTa tests of Latorre and Jones (1979)

clearly differentiated *Psm* race 1 ($G^-A^-T^+Ta^+$) and *Psm* race 2 ($G^+A^-T^-Ta^-$) isolates, but they had to be used in conjunction with the colour of growth in NSB to differentiate *Pss* ($G^+A^+T^-Ta^-$, yellow growth) and isolates with intermediate ($G^+A^+T^-Ta^-$, white or yellow/white growth) characteristics (Table 3). Agglutination and indirect-ELISA tests using three polyclonal antisera clearly distinguished most *Psm* race 1 and *Psm* race 2 from other *P. syringae* isolates; isolates with intermediate characteristics in biochemical and physiological tests were serologically similar to most *Psm* race 2 isolates. The serological tests also showed a range of variation amongst *Pss* isolates (Table 4).

Inoculation of micropropagated plantlets of lilac and wild cherry gave consistent results and confirmed the differentiation between *Pss* and *Psm* using biochemical, physiological and/or serological tests (Table 5). *Pss* isolates showed a range of variation in pathogenicity to lilac. The results of inoculations of rooted lilac plants correlated well with the results obtained with micropropagated lilac plantlets (isolates that were very aggressive in micropropagated lilac plants also produced lesions in older rooted plants), but micropropagated plantlets seem to be more susceptible than mature plants (Table 5).

Forty out of the 54 isolates categorised as *Pss* on the basis of biochemical and physiological tests were pathogenic on micropropagated plantlets of lilac (33 were also pathogenic on rooted lilac plants); therefore these isolates should be considered *Pss*; the remaining 14 isolates were not pathogenic on lilac and therefore should not be considered as *Pss* but as *P. syringae* (Table 6). Isolates that were strongly pathogenic on micropropagated lilac, but less aggressive on mature lilac and cherry (e.g. isolate HRI 5264) might constitute a different variant/race of *Pss*. The serological tests did not always distinguish between some very aggressive and some weak or non-pathogenic isolates (Tables 4–6). Nevertheless, all 40 isolates that were pathogenic on micropropagated lilac plantlets agglutinated with antiserum 00/9/3 and all, except one (isolate 5356A) agglutinated with 105D; 30 of these isolates did not agglutinate well with 00/8/3. In indirect-ELISA, 32 of these isolates were positive with antisera 00/9/3 and/or105D and 34 did not react with 00/8/3 (Table 6).

The *Psm* race 2 isolates and the isolates classified as intermediate on the basis of the biochemical and physiological tests were very uniform in serological and pathogenicity tests (Table 6) with exception of one isolate (HRI 5271) and therefore should all be included in *Psm* race 2. Isolates that agglutinated strongly with antiserum 00/8/3, but not with antisera 00/9/3 and 105D were all included in *Psm* race 1; two *Psm* race 1 isolates from plum also agglutinated well with 00/9/3. Although we did not observe leaf spots in all the plants inoculated with *Psm* races 1 and 2 isolates (Table 5), all these isolates are probably pathogenic. To check this hypothesis, more repetitions would be needed and sweet cherry cultivars should also be tested as wild cherry cultivars might be inherently more resistant. On the basis of biochemical, physiological and serological tests, *Psm* race 2 could be considered to be an intermediate form between *Pss* and *Psm* race 1.

Bacterial canker of wild cherry in England seems to be mainly caused by *Pss*, but can also be caused by *Psm* races 1 and 2. On the basis of biochemical, physiological and serological tests and pathogenicity tests on lilac and cherry, 26 of the isolates obtained from wild cherry with bacterial canker symptoms were assigned to *Pss*, five to *Psm* race 1 and 10 to *Psm* race 2 (including isolates with intermediate characteristics in biochemical and physiological tests). Studies in the UK by Garrett and Wood (unpublished) and Luz (1997) and in other European countries, South Africa and USA (Crosse and Garrett, 1963; Latorre and Jones, 1979; Roos and Hatting, 1986; Burkowicz and Rudolph, 1994) also indicated that both *Pss* and *Psm* can cause bacterial canker of wild and sour cherry, but they did not attribute so much importance to race 2 of *Psm*. In contrast, results obtained previously by Wormald (1937), Crosse (1955), Garrett et al. (1966) and Burkowicz and Rudolph (1994) indicated that *Psm* was the main cause of bacterial canker in sweet cherry and plum in England. Although our study did not include a large number of isolates from sweet cherry and plum, in our collection eight out of 22 isolates from sweet cherry and two out of seven isolates from plum were *Pss*, indicating that, in England, *Pss* can also cause bacterial canker in these hosts.

Wild cherry trees can have a diverse population of canker-causing bacteria. In two cases, isolates obtained in this study from the same plantation/nursery were assigned to different pathovars (and different serology groups): *Psm* race 2 (with intermediate characteristics in biochemical and physiological tests), *Pss* and *P. syringae* (not pathogenic on lilac) isolates were obtained from a single plantation in Essex; and *Pss* (two pathogenicity groups), *P. syringae*

Table 6. Distribution of isolates amongst groups based on combined results of serological, classical[a] and pathogenicity tests

Agglutination (score range[b]) with antisera			ELISA absorbances[b] with antisera			Numbers of isolates (and their hosts[c])							
						Pss group ($G^+A^+T^-Ta^-$, yellow in NSB) Pathogenicity[d] on rooted/micropropagated lilac				*Psm* race 2 group ($G^+A^-T^-Ta^-$, white in NSB)	Intermediate group ($G^+A^+T^-Ta^-$, white or yellow/ white in NSB)	*Psm* race 1 group ($G^-A^-T^+Ta^+$, white in NSB)	Others ($G^+A^-T^+Ta^-$, $G^-A^-T^-Ta^-$)
00/8/3	00/9/3	105D	00/8/3	00/9/3	105D	+/+ (*Pss*)	(+)/+ (*Pss*)	−/(+) (*Pss*)	−/− (*Ps*)				
0–1	3–4	3–4	<0.5	≥1.0	≥1.0	9 (w, s, p, cl)	2 (w)						
			<0.5	≥1.0	<1.0	5 (w, s)			1 (w)				
			<0.5	<1.0	≥1.0	5 (w, s, l)		4 (w)	2 (w)				
			<0.5	<1.0	<1.0	4 (w, s)			2 (w)				
3–4	3–4	3–4	≥1.0	<1.0	≥1.0	1 (s)	1 (w)		3 (w)				
			≥1.0	<1.0	<1.0	1 (pr)	1 (w)		1 (w)				
			≥1.0	≥1.0	≥1.5		1 (w)	1 (w)					
			<1.0	<1.0	≥1.0	1 (w)		1 (w)					
			<1.0	≤0.5	<1.0	1 (s)		1 (w)	1 (w)				
0–1	3–4	1–2	<0.5	≥1.0	<1.0	1 (s)							
			<1.0	<1.0	≥1.0				1 (w)				
			<1.0	<0.5	<1.0				1 (w), 1 (s)				
3	0	3	<0.5	<0.5	<0.5				1 (w)				
3–4	3–4	0–1	≥1.0	≤0.5	<1.0					7 (w, s)	8 (w)		
			<1.0	≤1.0	<0.5							2 (p)	
3–4	0–1	0–1	≥1.0	<1.0	<0.5							15 (w, s, p)	
0	0	1–2	<0.5	<0.5	≤1.0					1 (w)			2 (my, ph)
Total number of isolates						28	5	7	14	8	8	17	2

[a]GATTa tests (G, gelatin liquefaction; A, aesculin hydrolysis; T, tyrosinase activity; Ta, tartrate utilisation) and colour of growth in nutrient sucrose broth.
[b]Underlined values were considered positive.
[c]Hosts are indicated in brackets: w, wild cherry; s, sweet cherry; p, plum; cl, cherry laurel; l, lilac, pr, pear; my, myrobalan; ph, peach.
[d]+, highly pathogenic; (+), weakly pathogenic; −, not pathogenic.

(not pathogenic on lilac) and *Psm* race 2 isolates were obtained from a single nursery in Oxfordshire. *Pss* isolates obtained from cankers and leaf spots from the same plantation in Suffolk were also included in different pathogenicity and serology groups. Although three *Pss* isolates obtained from spots and cankers on sweet cherry in an orchard in Kent were all strongly pathogenic on cherry and lilac, the serology tests showed that they were also diverse. Nevertheless, the pathogenic populations of wild cherry seem to be more diverse than the populations found previously on sweet cherry. This difference may be due to the fact that sweet cherry is normally grown in orchards with a small number of cultivars (limited range of genotypes) and are clonally propagated by grafting and budding from only a few trees, whilst wild cherry is grown in more diverse habitats, includes a greater mixture of genotypes and is mainly grown in mixed plantations. The results of this study allow a rational selection of isolates for use in screening wild cherry for resistance to bacterial canker; at least one *Psm* race 1, one *Psm* race 2 and two *Pss* isolates representing the main groups established in this study should be included in future screens.

The *P. syringae* isolates obtained from wild cherry plant material can be discriminated using classical biochemical and physiological tests, but these tests have the disadvantage of being time-consuming and the distinction between *Pss* and some *Psm* isolates is not always simple due to intermediate types (Table 3). Our results clearly show that serological tests with two antisera produced against cherry isolates and an antiserum produced against a *P. syringae* isolate from pea could be used as an alternative to the classical tests to increase the speed of detection and discrimination of isolates. Agglutination tests with these antisera are especially indicated because they are very quick and seem to be sufficiently specific to differentiate *Pss* and *Psm* (Table 4), thus giving an early indication of the pathovar. The serological tests targeted the majority of pathogenic *Pss* isolates, but they did not always distinguish these isolates from other non-pathogenic *Ps* isolates (Table 6). Therefore, pathogenicity tests should always be performed to confirm the identification of *Pss* isolates. Pathogenicity tests using micropropagated plantlets have many advantages over inoculations in the field or inoculations of twigs or fruits: plantlets are easily multiplied and can be available all year round and this method is quicker, more consistent and clearly differentiates *Pss* and *Psm* isolates (Vicente and Roberts, 2002).

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