

TAXONOMY AND PATHOGENICITY OF *PSEUDOMONAS SYRINGAE* ISOLATES FROM CHERRY (*PRUNUS AVIUM*)

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Background

- Bacterial canker is one of the most important diseases of cherry (*Prunus avium* L.) and a major limitation for timber production from wild cherry.
- Most previous studies have been on sweet cherry, with *Pseudomonas syringae* pv. *morsprunorum* (*Psm*) considered the primary cause in the UK and both *P. syringae* pv. *syringae* (*Pss*) and *Psm* in other countries.
- More recently, *Pss* and/or intermediate forms between *Psm* and *Pss* were also found in sweet and in wild cherry.
- Our aim was to identify the pathogens associated with bacterial canker in wild cherry in England.



Methods

- 74 *Pseudomonas syringae* isolates from cherry (mostly from England 1957-2000) plus 13 others.
- Characterised by: Physiological / biochemical tests (fluorescence, colour in NSB, GATTa₂); Pathogenicity; Agglutination and ELISA (three antisera); rep-PCR.

Summary of physiological / biochemical, pathogenicity and agglutination test results.

Group	Fluor. ^a	Col. in NSB	G A T Ta ^b	No. Hosts ^c (no.)	Path on mature lilac	Path on micropropagated				Agglutination ^d			
						Lilac	Charger	1912	Spots	8/3	9/3	105D	
<i>Pss</i>	v	y	++--	28	w (14), s (8), cl (1), pl (2), l (2), pr (1)	+	+	+	+	-	+/-	+	+
				5	w (5)	(+)	+	+/-	+/-	-	+/-	+	+
				7	w (7)	-	+	+/-	+/-	-	+/-	+	+
				14	w (13), s (1)	-	-	-	-	-	+/-	+/-	+/-
<i>Psm</i> race 1	N	w	--++	10	w (4), s (5), p (1)	-	-	+	+/-	+ ^e	+	+/-	-
				7	w (1), s (2), p (4)	-	-	-	-	+ ^f	+	+/-	-
<i>Psm</i> race 2	v	w	++--	8	w (2), s (6)	-	-	+/-	+/-	+ ^g	+/-	+/-	-
Intermed-iate	B	w or yw	++--	8	w (8)	-	-	+/-	+/-	+ ^h	+	+	-
Others	N	y	++--	2	myr, (1), ph (1)	-	-	-	-	-	-	-	(+)

^a Fluorescence on King's medium B: v - variable; N - non-fluorescent, B - blue fluorescent

^b Gelatinase, Aesculin hydrolysis, Tyrosinase, Tartrate utilisation

^c Hosts: w, wild cherry; s, sweet cherry; p, plum; cl, cherry laurel; l, lilac, pr, pear; my, myrobalan; ph, peach.

^d Antiserum 8/3 prepared to *Psm* and 9/3 to *Pss* from wild cherry, 105D to *Ps* from pea.

^e Nine, ^f three, ^g six, and ^h four of these isolates produced leaf spots on plantlets of Charger and/or 1912.

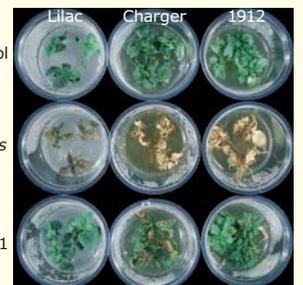
Conclusions

- Bacterial canker is present throughout southern England and can be caused by either *Psm* or *Pss*.
- The GATTa tests plus the colour of growth in NSB can differentiate *Psm* races 1 and 2 from other *P. syringae* isolates.
- Serological tests or rep-PCR can be used as alternatives to the classical tests to identify *Psm*, but cannot replace pathogenicity for *Pss*.

Pathogenicity

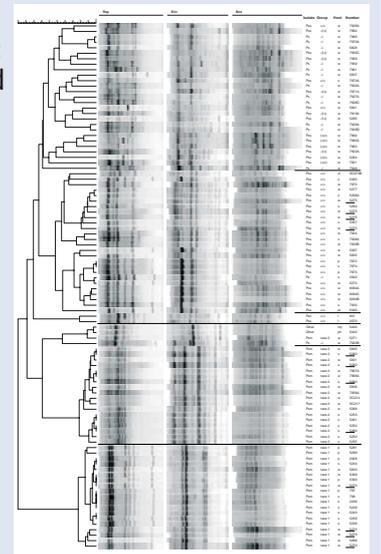
Tested on micropropagated lilac (Sensation) and two wild cherry clones (Charger and 1912) and rooted lilac cv. Sensation plants.

- Differentiated *Pss* and *Psm* isolates.
- Demonstrated a range of aggressiveness amongst *Pss* isolates.



rep-PCR

Total genomic DNA amplified using REP, ERIC and BOX primers.



- *Psm* race 1 uniform.
- *Psm* race 2 uniform.
- *Psm* races easily differentiated.
- *Pss* highly variable.



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Publications

- Vicente, J.G. and Roberts, S.J. (2006) Discrimination of isolates of *Pseudomonas syringae* from sweet and wild cherry using rep-PCR. *European Journal of Plant Pathology* (submitted).
- Vicente, J.G., Alves, J.P., Russell, K. and Roberts, S.J. (2004) Identification and discrimination of *Pseudomonas syringae* isolates from wild cherry in England. *European Journal of Plant Pathology* 110, 337-351.
- Vicente, J.G. and Roberts, S.J. (2003) Screening wild cherry micropropagated plantlets for resistance to bacterial canker. In: *Developments in Plant Pathology: Pseudomonas syringae pathovars and related pathogens* ed. Iacobellis, N.S. Dordrecht: Kluwer Academic Publishers.

Taxonomy and pathogenicity of *P.syringae* isolates from cherry (*Prunus avium*)

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Bacterial canker is one of the most important diseases of sweet and wild cherry (*Prunus avium* L.). This disease can be caused by two pathovars of *Pseudomonas syringae*: pv. *morsprunorum* (*Psm*) and pv. *syringae* (*Pss*). Seventy-four *Pseudomonas syringae* isolates from cherry and 13 isolates from other hosts were characterised by physiological, biochemical, serological and pathogenicity tests. Repetitive DNA polymerase chain reaction-based fingerprinting (rep-PCR) was also investigated as a method to distinguish pathovars, races and isolates. Physiological and biochemical tests discriminated *Psm* races 1 and 2 from other *P. syringae* isolates. Agglutination and indirect-ELISA 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. The results of rep-PCR using three sets of primers (REP, ERIC and BOX), indicated that the *Pss* isolates were highly variable, the two races of *Psm* can be easily separated and the *Psm* isolates are generally very uniform within each race. Serological tests or rep-PCR could be used as alternatives 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.