

COST 873, Angers, France, 17-19 April 2007

## ***Pseudomonas syringae* associated with bacterial canker in England**

Steven Roberts<sup>1</sup> & Joana Vicente<sup>2</sup>

<sup>1</sup>Plant Health Solutions - [www.planthealth.eu](http://www.planthealth.eu)

<sup>2</sup>Warwick-HRI, UK



### Outline

- Background
- Biochemical tests
- Serology
- Pathogenicity
- rep-PCR

©2007 PLANT HEALTH SOLUTIONS



### Bacterial canker

- First came across bacterial canker ~25 yrs ago
- Long term interest
- Little funding



©2007 PLANT HEALTH SOLUTIONS



### Bacterial canker



©2007 PLANT HEALTH SOLUTIONS



### Bacterial canker & Farm Woodlands

- Cherry important in farm woodlands in UK
  - amount of cherry that can be planted is restricted due to canker
  - growers want to plant more
  - resistance sought, but which strains to use for screening ?
- Our main aim was to identify the pathogens associated with wild cherry in England

©2007 PLANT HEALTH SOLUTIONS



### Bacterial canker

- Can be caused by two pathovars of *Pseudomonas syringae*:
  - pv. *morsprunorum* (*Psm*) and pv. *syringae* (*Pss*).
- Traditionally in the UK considered to be mainly caused by *Psm* in sweet cherry
- 1975 – new variant of *Psm* (designated race 2) that showed distinct pathogenicity to some cherry cultivars was identified at East Malling

©2007 PLANT HEALTH SOLUTIONS



## Isolates

- Sweet and Wild Cherry
  - from East Malling collection (ex Garrett, Billing, Crosse)
  - obtained as part of disease survey in Wild cherry
- Other hosts
  - from HRI collection and others
- Characterised on the basis of physiological & biochemical tests, serology, pathogenicity, rep-PCR



©2007 PLANT HEALTH SOLUTIONS

## Physiological/Biochemical tests

- GATTa tests
  - G, gelatin liquefaction
  - A, aesculin hydrolysis
  - T, tyrosinase activity
  - Ta, tartrate utilisation
  - Colour of growth in nutrient sucrose broth (NSB)
  - Fluorescence on King's B (KB) medium



©2007 PLANT HEALTH SOLUTIONS

## Physiological/Biochemical tests

Group	Fluor.	NSB	GATTa <sup>a</sup>	No.	Hosts
<i>Pss</i>	v	y	++--	14	w, s, cl, p, l, pr
<i>Ps</i>	v	y	++--	14	w, s
<i>Psm</i> race 1	N	w	--++	10	w, s, p
<i>Psm</i> race 2	v	w	----	8	w, s
'Intermediate'	B	w or yw	++--	8	w
Others	N	w or y	+-+ or ----	2	myr, ph

<sup>a</sup> Gelatinase, Aesculin, Tyrosinase, Tartrate  
Hosts: wild cherry, sweet cherry, cherry laurel, plum, lilac, pear, myrobalan, peach



©2007 PLANT HEALTH SOLUTIONS

## Physiological/Biochemical tests

- GATTa tests plus the colour of growth in NSB can differentiate *Psm* races 1 and 2 from other *P. syringae* isolates



©2007 PLANT HEALTH SOLUTIONS

## Serology

- Agglutination and indirect-ELISA
- Three different polyclonal antisera
  - *Psm* race 1 (08/03)
  - *Pss* from wild cherry (09/03)
  - *P. syringae* from pea (105D)



©2007 PLANT HEALTH SOLUTIONS

## Serology

Group	08/03	09/03	105D	No.	Hosts
<i>Pss</i>	+/-	+	+	14	w, s, cl, p, l, pr
<i>Ps</i>	+/-	+/-	+/-	14	w, s
<i>Psm</i> race 1	+	+/-	-	10	w, s, p
<i>Psm</i> race 2	+	+	-	8	w, s
'Intermediate'	+	+	-	8	w
Others	-	-	(+)	2	myr, ph



©2007 PLANT HEALTH SOLUTIONS

## Serology

- *Psm* race 1 and race 2 were relatively uniform
- *P. syringae* very variable
- Pathogenic *Pss* isolates could not be distinguished from non-pathogenic isolates of *P. syringae*
- Agglutination tests can be used as a quick alternative to classical tests
  - give a early indication of the pathovar
  - do not always distinguish the pathogenic *Pss* from other non-pathogenic *P. syringae* isolates



©2007 PLANT HEALTH SOLUTIONS

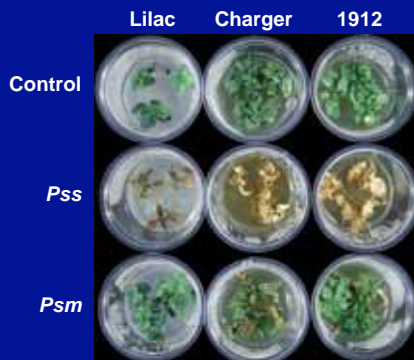
## Pathogenicity

- Inoculations on:
  - rooted lilac cv. Sensation
  - micropropagated lilac plantlets (cv. Sensation)
  - two micropropagated wild cherry clones (cv. Charger and accession 1912)



©2007 PLANT HEALTH SOLUTIONS

## Pathogenicity



©2007 PLANT HEALTH SOLUTIONS

## Pathogenicity

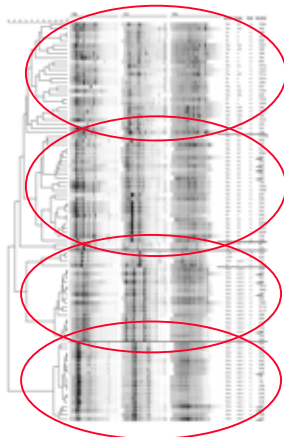
- Clearly differentiated *Pss* and *Psm* isolates
- Demonstrated a range of aggressiveness amongst *Pss* isolates
- Inoculation of microprop. plantlets gave more consistent and reproducible results than inoculation of twigs



©2007 PLANT HEALTH SOLUTIONS

## rep-PCR

- Used REP, ERIC and BOX primers,
- Several clearly defined groups:
  - *Ps* + *Pss*
  - *Pss*
  - *Psm* Race 2 + 'Intermediates'
  - *Psm* Race 1



©2007 PLANT HEALTH SOLUTIONS

## rep-PCR

- *pv. syringae* isolates were highly variable.
- *pv. morsprunorum* race 1 isolates were very uniform
- *pv. morsprunorum* race 2 isolates were also very uniform and distinct from the race 1 isolates
- 'Intermediate' types were grouped with *Psm* race 2



©2007 PLANT HEALTH SOLUTIONS

## rep-PCR

- Can be used to identify isolates of the two *Psm* races
- Can assist in the identification of *Pss* isolates but cannot replace inoculation of susceptible hosts like lilac

©2007 PLANT HEALTH SOLUTIONS



## Conclusions

- Survey: bacterial canker is present throughout England
  - considered a permanent threat to sweet and wild cherry production
  - Both *Psm* and *Pss* can be found causing cankers in wild cherry
- 'Intermediates' should be considered as *Psm* race 2
- *Psm* races genetically distinct and very uniform

©2007 PLANT HEALTH SOLUTIONS



## Conclusions

- Serological tests or rep-PCR can be used as alternatives to classical tests to identify and discriminate *Psm* isolates, but pathogenicity tests still necessary to discriminate pathogenic *Pss* isolates.

©2007 PLANT HEALTH SOLUTIONS



## Comment on *pv. avii*

- In our pathogenicity tests *pv. avii* behaved the same as *pv. morsprunorum* (i.e. leaf spots on cherry, nothing on lilac)
- Genetically distinct from *Psm* race 1 and race 2
- BUT appears to be no more different from *Psm* races 1 and 2 than race 1 to race 2 and closer to race 2 than race 1
- We do not believe the creation of this new *pv.* is justified – it is not distinctly pathogenic from *Psm* race 2 and is classified in the species *P. syringae*
- Arguably more justification to move *Psm* race 1 into *P. savastanoi*?

©2007 PLANT HEALTH SOLUTIONS



## Acknowledgements

- This work was funded in the UK by Defra

©2007 PLANT HEALTH SOLUTIONS



The End  
Thankyou for listening

Steve Roberts  
E: [s.roberts@planthealth.eu](mailto:s.roberts@planthealth.eu)  
W: [www.planthealth.eu](http://www.planthealth.eu)

©2007 PLANT HEALTH SOLUTIONS

