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

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

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

**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

**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
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

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

Physiological/Biochemical tests

<table>
<thead>
<tr>
<th>Group</th>
<th>Fluor</th>
<th>NSB</th>
<th>GATTa</th>
<th>No.</th>
<th>Hosts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pss</td>
<td>v</td>
<td>y</td>
<td>+ + – –</td>
<td>14</td>
<td>w, s, cl, p, l, pr</td>
</tr>
<tr>
<td>Ps</td>
<td>v</td>
<td>y</td>
<td>+ + – –</td>
<td>14</td>
<td>w, s</td>
</tr>
<tr>
<td>Psm race 1</td>
<td>N</td>
<td>w</td>
<td>– – + +</td>
<td>10</td>
<td>w, s, p</td>
</tr>
<tr>
<td>Psm race 2</td>
<td>v</td>
<td>w</td>
<td>+ – – –</td>
<td>8</td>
<td>w, s</td>
</tr>
<tr>
<td>Intermediate</td>
<td>B</td>
<td>w or y</td>
<td>+ + – –</td>
<td>8</td>
<td>w</td>
</tr>
<tr>
<td>Others</td>
<td>N</td>
<td>w or y</td>
<td>+ – + – or 2</td>
<td>myr, ph</td>
<td></td>
</tr>
</tbody>
</table>

* Gelatinase, Aesculin, Tyrosinase, Tartrate
Hosts: wild cherry, sweet cherry, cherry laurel, plum, lilac, pear, myrobalan, peach

Physiological/Biochemical tests

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

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)

Serology

<table>
<thead>
<tr>
<th>Group</th>
<th>08/03</th>
<th>09/03</th>
<th>105D</th>
<th>No.</th>
<th>Hosts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pss</td>
<td>+ / –</td>
<td>+</td>
<td>+</td>
<td>14</td>
<td>w, s, cl, p, l, pr</td>
</tr>
<tr>
<td>Ps</td>
<td>+ / –</td>
<td>+ / –</td>
<td>+ / –</td>
<td>14</td>
<td>w, s</td>
</tr>
<tr>
<td>Psm race 1</td>
<td>+</td>
<td>+ / –</td>
<td>–</td>
<td>10</td>
<td>w, s, p</td>
</tr>
<tr>
<td>Psm race 2</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>8</td>
<td>w, s</td>
</tr>
<tr>
<td>Intermediate</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>8</td>
<td>w</td>
</tr>
<tr>
<td>Others</td>
<td>–</td>
<td>–</td>
<td>(+)</td>
<td>2</td>
<td>myr, ph</td>
</tr>
</tbody>
</table>
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 an early indication of the pathovar
  - do not always distinguish the pathogenic *Pss* from other non-pathogenic *P. syringae* isolates

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

Pathogenicity

<table>
<thead>
<tr>
<th>Lilac</th>
<th>Charger</th>
<th>1912</th>
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<tbody>
<tr>
<td>Control</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
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<tr>
<td><em>Pss</em></td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
<tr>
<td><em>Psm</em></td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
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</tbody>
</table>

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

Pathogenicity
- *Pss* 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 1

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

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

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.

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*?

Acknowledgements
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The End
Thankyou for listening

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