Variability in the Nordic *Phytophthora infestans* population


GILB 2008
When did late blight arrive in the Nordic countries?

- Norway 1841 (Svensen, 1852)
- Sweden 1845 (Wahlberg, 1847)
- Denmark 1845 (Brøndegaard ?)
- Finland 1847 (Mäkelä 1966)
When was mating type A2 first found in the Nordic countries?

- Sweden 1985
  (Kadir & Umaerus, 1987)
- Finland 1992
- Norway 1993
  (Hermansen et al. 2000)
- Denmark 1996
  (Bødker et al. 1998)
- Iceland 1999
  (Olafsson & Hermansen 2001)
Results from *P. infestans* population studies in Finland and Norway in the 1990`s

- Mating type: Finland -15 % A2 and Norway - 25 % A2, both mating types found in 34-64 of the fields
- Metalaxyl resistance was common
- All known virulence genes were found
- Genotypes (RG57): 76 genotypes found among 141 isolates, 53 were detected once => *Indication of sexual reproduction*
- Oospores were observed in potato leaves

*(Brurberg et al., 1999, Hermansen et al. 2000)*
Norphyt - project 2003-2006

Studies on the new Nordic populations of *P. infestans* to improve potato late blight forecasting and control
Collection of NorPhyt blight isolates in 2003

- 75 single lesion isolates from 75 separate fields in DK, FIN, N and SE was aimed to be collected
- Total number of 100-300 isolates were sampled except in DK only 63 isolates
- Isolates were transferred to pea agar via tuber slices and kept on pea or rye agar until further studies
Characterisation of NorPhyt isolates

- **Phenotypic traits**
  - Mating type (all collected isolates in each country)
  - Response to metalaxyl and propamocarb (FIN)
  - Virulence spectrum based on Black’s R-gene differential set obtained from SASA (50 isolates/country) (FIN)  
    (Lehtinen et al. 2008, Plant Pathology 57, 227-234)

- **Foliar aggressiveness traits** (25 isolates/country) (FIN, NO, SE)
  - to see the variation in current population
  - to improve validity of epidemiological models and DSS’s
    (Lehtinen et al. 2008, Plant Pathology, accepted)

- **Genotypic traits** (50 isolates/country) (N)
  - Microsatellites (SSR)
    (Brurberg et al. 2008, in prep)
Phenotypic characterisation

• Mating type distribution
Phenotypic characterisation

- Mating type distribution

% of fields

<table>
<thead>
<tr>
<th></th>
<th>FIN</th>
<th>NO</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of fields</td>
<td>34%</td>
<td>28%</td>
<td>7%</td>
</tr>
<tr>
<td>both mating types</td>
<td>37</td>
<td>28</td>
<td>7</td>
</tr>
<tr>
<td>one mating type</td>
<td>34</td>
<td>79</td>
<td>10</td>
</tr>
</tbody>
</table>
Phenotypic characterisation

• Response to metalaxyl

% of Isolates sporulating

<table>
<thead>
<tr>
<th></th>
<th>DK</th>
<th>FIN</th>
<th>N</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 %</td>
<td>2</td>
<td>2</td>
<td>6</td>
<td>13</td>
</tr>
<tr>
<td>80 %</td>
<td>5</td>
<td>6</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>60 %</td>
<td>23</td>
<td>226</td>
<td>53</td>
<td>63</td>
</tr>
<tr>
<td>40 %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Metalaxyl
- 100 mg/l
- 10 mg/l
- 1 mg/l
- 0 mg/l
Phenotypic characterisation
• Response to propamocarb hydrochloride

% of Isolates sporulating

<table>
<thead>
<tr>
<th></th>
<th>0 mg/l</th>
<th>10 mg/l</th>
<th>100 mg/l</th>
<th>1000 mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>DK</td>
<td>6</td>
<td>23</td>
<td>19</td>
<td>7</td>
</tr>
<tr>
<td>FIN</td>
<td>13</td>
<td>12</td>
<td>22</td>
<td>75</td>
</tr>
<tr>
<td>N</td>
<td>12</td>
<td>10</td>
<td>2</td>
<td>49</td>
</tr>
<tr>
<td>SE</td>
<td>12</td>
<td>10</td>
<td>2</td>
<td>49</td>
</tr>
</tbody>
</table>

Propamocarb concentrations:
- 1000 mg/l
- 100 mg/l
- 10 mg/l
- 0 mg/l
Phenotypic characterisation

- Virulence spectrum

Proportion of tested isolates able to overcome different R-genes
**Phenotypic characterisation**

- **Virulence spectrum**

<table>
<thead>
<tr>
<th>Pathotype</th>
<th>DK</th>
<th>FIN</th>
<th>N</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1,3,4,7,10,11</td>
<td>38.5</td>
<td>42.9</td>
<td>60.0</td>
<td>10.9</td>
</tr>
<tr>
<td>R1,2,3,4,7,10,11</td>
<td>5.1</td>
<td>21.4</td>
<td>0.0</td>
<td>21.7</td>
</tr>
<tr>
<td>R1,2,3,4,6,7,10,11</td>
<td>17.9</td>
<td>2.4</td>
<td>4.0</td>
<td>8.7</td>
</tr>
<tr>
<td>R1,3,4,6,7,10,11</td>
<td>15.4</td>
<td>0.0</td>
<td>2.0</td>
<td>6.5</td>
</tr>
<tr>
<td>R1,3,4,7,11</td>
<td>0.0</td>
<td>7.1</td>
<td>6.0</td>
<td>6.5</td>
</tr>
<tr>
<td>R1,3,4,5,7,10,11</td>
<td>0.0</td>
<td>7.1</td>
<td>8.0</td>
<td>2.2</td>
</tr>
<tr>
<td>R1,2,3,4,5,6,7,10,11</td>
<td>7.7</td>
<td>0.0</td>
<td>4.0</td>
<td>2.2</td>
</tr>
<tr>
<td>R1,3,4,7,8,10,11</td>
<td>0.0</td>
<td>7.1</td>
<td>6.0</td>
<td>0.0</td>
</tr>
<tr>
<td>R1,3,4,5,6,7,8,10,11</td>
<td>0.0</td>
<td>0.0</td>
<td>2.0</td>
<td>4.3</td>
</tr>
<tr>
<td>R1,4,7,10,11</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>6.5</td>
</tr>
</tbody>
</table>

Ten most common types covered 85% of the population. The rest 23 types were found once or twice.

Pathotype complexity (virulence factors per isolate):
- **S** 6.87
- **DK** 6.92
- **FIN** 6.40
- **N** 6.26
Foliar aggressiveness

• Tested on Bintje and Matilda in the lab
• Measurements
  • Leaf disks
    • Infection Efficacy (% infectious sporangia)
  • Detached leaves
    • Latency period (hours)
    • Lesion growth rate (mm/day)
    • Spore production/lesion 7 (9) days after inoculation
Latency period
Lesion growth rate

(a) Bintje

(b) Bintje

(c) Bintje

(d) Matilda

(e) Matilda

(f) Matilda
Foliar aggressiveness

- Differences between countries insignificant
- Within country variation substantial

- Infection frequency < 1%
- Latency period 80 - 180 hours
- Lesion growth 0 - 8 mm/day
- Sporulation capacity 0 - 1300 sporangia mm$^{-2}$

- Differences between test laboratories significant
Genotypic traits

200 Nordic isolates selected for genotyping with SSR

50 from each country (N, S, FIN, DK)
SSR analysis

• **SSR markers used**
  - Pi4B, Pi4G, PiG11 (Knapova & Gisi)
  - Pi02, Pi04, Pi16, Pi26, Pi33 (Lees & Cooke)
  - D13 (Pipe & Shaw)

• **ABI 3730 DNA Analyzer (capillary system) for fragment separation**
  - **Software**
    - Data Collection v 2.0
    - GeneMapper v 3.0

  Used 10 of SCRIIs control isolates to fine tune the system
### SSR analysis

**Alleles found in the Nordic population**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Allele size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pi02</td>
<td>142 150 152 156 158 160 162 164 166</td>
</tr>
<tr>
<td>Pi04</td>
<td>160 162 166 168 170 172</td>
</tr>
<tr>
<td>Pi16</td>
<td>160 166 168 172 176 178</td>
</tr>
<tr>
<td>Pi26</td>
<td>171 173 177 179 181 183 185 187</td>
</tr>
<tr>
<td>Pi33</td>
<td>203 206 209</td>
</tr>
<tr>
<td>D13</td>
<td>106 108 116 118 132 134 136 142</td>
</tr>
<tr>
<td>4B</td>
<td>203 205 213 217</td>
</tr>
<tr>
<td>4G</td>
<td>160 162 164 166 168 170 174 176</td>
</tr>
<tr>
<td>G11</td>
<td>140 142 148 150 152 154 156 158 160 162 166</td>
</tr>
</tbody>
</table>
Genetic variation - SSR markers

• 190 genotypes (200 isolates)
  Based on only 7 SSR markers
• 6 genotypes occurred twice
• 1 genotype occurred six times  (4 N, 2 Fi)
Conclusions regarding the current Nordic *P. infestans* population (NorPhyt)

- Both mating types common in all Nordic countries - but some regional variation
- The majority of the population are metalaxyl- and propamocarb sensitive
- Complex virulence races are dominating
- The population show high genetic variation (SSR-data) indicating sexual reproduction
- Large differences in foliar aggressiveness among isolates
- No need for individual parameterization of DSS submodels for each Nordic country
THANK YOU FOR YOUR ATTENTION!