Eucablight – one year on: an update on European blight populations

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Poul Lassen
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All data submitters
Overview

• Introduction – Eucablight project aims

• What elements of *P. infestans* biology are important

• What contributions Eucablight pathogen database can (and cannot) make

• Eucablight overview
  • data collection & progress since Tallinn
  • need for pathogen genotyping (SSR) data
  • data interpretation and presentation

• Key examples of the data collected

• Conclusions & future plans
Main aims of pathogen section of Eucablight project

**WP1**
‘... establish a **comprehensive network** on the population biology of *P.infestans* across Europe’

**WP2**
**Collect and collate data** (pheno and genotype) on existing/past *P. infestans* collections

**WP4**
**4.1 Collate and review existing methods** for assessing variation in *P. infestans* populations and to test, standardise and publish these methods in a www database

**4.2** To create a **European isolate database** detailing existing data on isolate variation using new data as assessed by the methods developed in Objective 4.1

**4.3** **Training** course on agreed and adopted methods

**4.4** Pan-European **interpretation** of changing population structure in *P. infestans*

**WP5**
**Integration of all derived data to benefit of control strategies**
Linking *P. infestans* biology and blight management

1. Where, when and how blight infection starts
   - primary inoculum

2. Rate of infection and spread
   - foliar
   - tuber

3. Control options
   - fungicide efficacy
   - host resistance

4. Survival
   - cull piles
   - volunteers
   - solanaceous weeds
   - oospores

5. Changing pathogen population
   - Immigration
   - Evolution – mutation & recombination

Integration of all data into practical management advice
**P. infestans VARIATION - Type**

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mating type</td>
<td>Isozyme</td>
</tr>
<tr>
<td>Virulence</td>
<td>RG57</td>
</tr>
<tr>
<td>Fungicide resistance</td>
<td>mtDNA</td>
</tr>
</tbody>
</table>

**Phenotype**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Isotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSR</td>
<td>mtDNA</td>
</tr>
<tr>
<td>AFLP</td>
<td>SNP</td>
</tr>
<tr>
<td>SNP</td>
<td>SEQ</td>
</tr>
</tbody>
</table>
## VARIATION - Other factors

<table>
<thead>
<tr>
<th>Host</th>
<th>Tomato</th>
<th>Potato</th>
<th>Others</th>
<th>Weeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Management</td>
<td>Variety</td>
<td>Fungicide</td>
<td>Crop type</td>
<td>Seed trade</td>
</tr>
</tbody>
</table>
Data collected -3

VARIATION - Scales

<table>
<thead>
<tr>
<th></th>
<th>Fine Scale</th>
<th>Coarse Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spatial</td>
<td>Leaf</td>
<td>Continent</td>
</tr>
<tr>
<td>Temporal</td>
<td>Weeks</td>
<td>Years</td>
</tr>
</tbody>
</table>
Data analysis options

Multiple options for data analysis
Depends on how complete the data set is

Fundamental and applied biology
Linkage between two is strong

Scales

Type
Other factors
Pathogen database

"Old" pathogen data

"New" pathogen data

Server at DIAS
Pathogen data overview

www.eucablight.org

Tallinn 12,300
Oct 2005

Rennes 13,600
Jan 2006

NJF 15,000
Nov 2006

Bologna 15,500
May 2007
Tools for estimating genetic diversity

Isozymes

RG57 RFLP fingerprint

mtDNA haplotype

38Kb
95% coding
Slow evolving
4 haplotypes world-wide

Forbes et al 1998

Brurberg et al 1999

AFLPs

Brurberg et al 1999
Simple Sequence Repeats

TCGACCACCGGTNNCCACCGTCGGGAAGCAGGCCTGGTGAAGACGATCA

Fwd Primer

TGCTAGGTCTGAGACTTGC

AGAACTACCGCCCGAGAC

AATTCGACCGAGCGGTGTAG

SSR 10 X 'TG' repeat

CTGAGTACTACTACGGAGCTTTGAGAGAGAGAGAGAGAGAGAGCTGCTTC

Rev Primer

GTGGTCTTCGCGCACCTTGCGCTCGTACAAGATGGTGGA

ATGTTCTTGTGACCATCC

AGAACTACCGCCCGAGAC

AATTCGACCGAGCGGTGTAGCTGAGTACTACTACGGAGCTTTGCTGCTTC

Accurate sizing

n.b diploid

11 markers/loci = (many potential combinations!)

Important features

- Similar to human forensics
- Objective – easy to compare lab to lab
- Specific
- Both alleles scored
- Can be run on leaf material
- Good resolution

PCR amplification

e.g. 162 bp product

11 markers/loci = (many potential combinations!)
How does SSR data look in practice?

<table>
<thead>
<tr>
<th>Outbreak 1</th>
<th>Outbreak 2</th>
<th>Outbreak 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Seed</td>
<td>Krue</td>
<td>M. Piper</td>
</tr>
<tr>
<td>2. Seed</td>
<td>Krue</td>
<td>M. Piper</td>
</tr>
<tr>
<td>3. Seed</td>
<td>Krue</td>
<td>M. Piper</td>
</tr>
<tr>
<td>4. Seed</td>
<td>Krue</td>
<td>M. Piper</td>
</tr>
<tr>
<td>5. Seed</td>
<td>Krue</td>
<td>M. Piper</td>
</tr>
<tr>
<td>6. Seed</td>
<td>Krue</td>
<td>M. Piper</td>
</tr>
<tr>
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<tbody>
<tr>
<td>8. Seed</td>
<td>Krue</td>
<td>M. Piper</td>
</tr>
<tr>
<td>9. Seed</td>
<td>M. of Galle</td>
<td>M. Piper</td>
</tr>
<tr>
<td>10. Seed</td>
<td>M. of Galle</td>
<td>M. Piper</td>
</tr>
<tr>
<td>11. Seed</td>
<td>M. of Galle</td>
<td>M. Piper</td>
</tr>
<tr>
<td>12. Seed</td>
<td>M. of Galle</td>
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</tr>
<tr>
<td>13. Seed</td>
<td>M. of Galle</td>
<td>M. Piper</td>
</tr>
<tr>
<td>14. Seed</td>
<td>M. of Galle</td>
<td>M. Piper</td>
</tr>
<tr>
<td>15. Seed</td>
<td>M. of Galle</td>
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</tr>
<tr>
<td>16. Seed</td>
<td>M. of Galle</td>
<td>M. Piper</td>
</tr>
<tr>
<td>17. Seed</td>
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<tr>
<th>Outbreak 1</th>
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<th>Outbreak 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>18. Ware</td>
<td>Spruelan</td>
<td>P. Javelin</td>
</tr>
<tr>
<td>19. Ware</td>
<td>Spruelan</td>
<td>P. Javelin</td>
</tr>
<tr>
<td>20. Ware</td>
<td>Spruelan</td>
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</tr>
<tr>
<td>21. Ware</td>
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<td>24. Ware</td>
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</tr>
<tr>
<td>25. Ware</td>
<td>Spruelan</td>
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Main aspects

1. Where, when and how blight infection starts
   - primary inoculum

2. Rate of infection and spread
   - foliar
   - tuber

3. Control options
   - fungicide efficacy
   - host resistance

4. Survival
   1. cull piles
   2. volunteers
   3. solanaceous weeds
   4. oospores

5. Changing pathogen population?
   1. Immigration
   2. Evolution
Distribution of mating types

Recent increases in A2
- N. France – 2005
- Netherlands - 2005
- UK – 2005 & 2006

Potential or real threat?
- Can the A1-A2 types mate?
- Role as primary inoculum?
- What types of A1 and A2 present?
Evidence of oospore derived epidemics?

Björn Andersson (SLU, Sweden)

Hannukala et al Finland (Plant. Path. 2007)
Main aspects

1. Where, when and how blight infection starts
   - primary inoculum

2. Rate of infection and spread – *not covered in eucablight*
   - foliar
   - tuber

3. Control options
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4. Survival
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5. Changing?
   1. Immigration
   2. Evolution
Metalaxyl resistance

Frequency of isolates by Metalaxyl resistance [All countries - All years]

- Resistant
- Intermediate
- Sensitive

Number of isolates: 7106

Frequency of isolates by Metalaxyl resistance [Norway - All years]

- Resistant
- Intermediate
- Sensitive

Number of isolates: 857
Metalaxyl resistance in relation to metalaxyl application

Frequency of isolates for Metalaxyl resistance by Contains Phenylamide [All countries - All years]

Frequency of isolates for Metalaxyl resistance by Contains Phenylamide [Northern Ireland - All years]

Total number of isolates: 175

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Metalaxyl resistance vs mating type

First chart: Frequency of isolates for Metalaxyl resistance by Mating type [All countries - All years]

- **A1**: n=5508
- **A2**: n=5338
- **SF**: n=171

Second chart: Frequency of isolates for Metalaxyl resistance by Mating type [Poland - All years]

- **A1**: n=82
- **A2**: n=44
- **SF**: n=2

Total number of isolates: 6617

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Could R-genes be used to control blight?
Not 1,3,4,7,10,11

More research on others (R5,R6,R8,R9) needed to understand why virulence frequency lower.
Main aspects

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   - Immigration
   - Evolution – mutation & recombination
SSR allele distribution
Regional patterns in populations (allele frequencies)

### Allele frequency for marker D13 by country for all years

- **England**
  - $N_{\text{isolate}} = 200$

- **Scotland**
  - $N_{\text{isolate}} = 717$

- **Wales**
  - $N_{\text{isolate}} = 32$

- **Northern Ireland**
  - $N_{\text{isolate}} = 146$

- **Netherlands**
  - $N_{\text{isolate}} = 24$

- **Belgium**
  - $N_{\text{isolate}} = 30$

- **Hungary**
  - $N_{\text{isolate}} = 154$

- **Sweden**
  - $N_{\text{isolate}} = 61$

- **Estonia**
  - $N_{\text{isolate}} = 25$
SSR allele change

![Bar chart showing SSR allele change with data points for SC 1995-7, SC 2003, SC 2004, and NL 2000(70).]
System updates

• Expansion to include South and Central America

• Improvements to efficiency of data transfer

• Addition of sequence data option
Conclusions & Future plans

• Unique resource to help understand pathogen population change on a range of scales (thanks to all data submitters and DIAS)
• *P. infestans* population differs from country to country
• Association between factors observed (e.g. fungicide resistance and mating type)
• New insights into pathogen change emerging

• Database updates and more interpretation at local and EU scale required
• Need to link data on population change with the cause of that change. Identify factors that ‘push’ or ‘pull’ population change (e.g. increased aggressiveness and fitness?).
• Exploitation of host resistance (GM-based?) – success of such a strategy will depend on understanding pathogen populations
• Expanding dataset beyond Europe to set context of EU populations