Euroblight analysis of *Phytophthora infestans* populations in Europe and expansion of the global database

David Cooke

Jens Hansen, Poul Lassen, Sanmohan Baby, Alison Lees, Geert Kessel

Thanks – industry, data contributors, ALAP & Tizon for invitation to Panama

Technical support; Louise Sullivan, Eva Randall, Trudy van den Bosch and Marieke Förche
Overview

- Context of research
- What is Euroblight?
- *P. infestans* genetics
- How we monitor EU populations
- Database structure and analysis tools
- Results
- Future directions - discussion
Why study populations?

- Disease Management
- Environ. Temp & Humidity
- Fungicide resistance
- Rate of evolution (mating)
- Survival & Spread
- Fitness Aggress. Virulence

Disease Management
Born from EU.ICP.NET and Eucablight projects
Informal consortium of research, commercial and extension staff
Industry-sponsored meetings - research funded via other means

Huub Schepers
Jens G Hansen
Alison Lees
Euroblight isolate database

- Hosted and maintained at Arhus, Denmark
- Same structure as Cereal rust database – expandable
- Holds information on more than 28,000 *P. infestans* isolates
- Data entry via MS-Excel templates
- Interface via Blight Toolbox. Username and password provided once data supplied
# Euroblight isolate database: Phase 1 2003-2006 – 25K isolates

## POTATO LATE BLIGHT TOOLBOX

- **Home**
- **Isolates**
- **Partners**

### Under construction

### SAMPLE MANAGEMENT

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<th>Collections and samples</th>
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SSR fingerprinting background

- Markers produced by 3 teams from 2000 onwards
  - Knapova and Gisi 2001
  - Lees et al SCRI 2006
  - Li and van der Lee 2010

- Some more useful than others

- Reviewed all markers & 12 most useful combined as 12-plex assay in single PCR (Li et al. 2013)

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<td>SSR7</td>
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</table>
Monitoring methods

- Scouts (advisers, industry, scientists) given FTA cards & sample forms (live cultures - FAB)
- Outbreak data (e.g. location, crop type, cultivar) recorded
- Lesions pressed onto FTA cards to capture pathogen DNA
- DNA fingerprinted using 12-plex SSRs (Li et al. 2013)
- Genotypes defined by DNA fingerprint and data uploaded to database via web interface (Arhus University)
- Data mapped on www.euroblight.net

SSR loci (1-12) showing allele peaks and size markers (orange)
Monitoring blight activity (Team GB)

- AHDB Potatoes ‘Fight Against Blight’ campaign
- Approx. 2000 outbreaks & 7000 isolates to date
- Samples sent to Hutton to isolate and genotype
EU Sampling 2013-2015

- 2982 samples from 30 countries genotyped
- Most samples from north-western Europe
- Long ‘tail’ of countries with few samples

Countries with >30 samples shown:
- EN – England
- FR – France
- SC – Scotland
- BE – Belgium
- NL – the Netherlands
- DE – Germany
- WA – Wales
- ND – Northern Ireland
- SE – Sweden
- PL – Poland
- DK – Denmark
- ES – Spain
- SI – Slovenia
- BY – Belarus
- CH – Switzerland

Sample number
EU P. infestans genotype change

- ~70% of population clonal
- EU_13_A2 dominant followed by EU_6_A1, EU_8_A1 and EU_1_A1
- EU_33_A2 ‘green 33’ very low frequency
- EU_36_A2 novel clone in 2014 and 2015
- Most inoculum survives overwinter in tubers
- 20-30% of population each year genetically diverse ‘Other’ group probably from oospores
2013 samples

- 733 samples from 27 countries
2014 samples

- 1545 samples from 24 countries
2015 samples

• 614 samples from 16 countries
2015 samples – EU_13_A2

Live map at http://euroblight.net/pathogen-characteristics-and-host-resistance/sampling-sites-and-genotype-maps/
2013-2015 summary

- 2892 samples from 30 countries
- EU_13_A2 clone most widespread
- Other clones clustered, e.g.
  - EU_6_A1 in UK and France
  - EU_8_A1 in Northern Ireland
  - EU_1_A1 in France and Belgium
- Higher proportion of ‘Other’ types in northern and eastern Europe
EuroBlight Population Genetics Platform

Potato Late Blight Toolbox

Population Statistics

Minimum Spanning Tree

Phylogenetic Tree

Principal Component Analysis

Zhian Kamvar & Nik Grünwald
Kamvar et al. 2015. Frontiers in Genetics 6:208
POLYSAT Bruvo distance analysis of international population (n=102)

- Discrimination at international level is robust
Toolbox interface

Potato Late Blight Toolbox

Isolate Statistics
Under construction

Year
[ ] 2005 [ ] 2004 [ ] 2002

 Continent
 [ ] Europe [ ] Africa & West/Central Asia [ ] East Asia [ ] South America

 Country
 [ ] All [ ] Belarus [ ] Belgium [ ] Bulgaria [ ] Croatia [ ] Cyprus [ ] Czech Republic [ ] Denmark [ ] England [ ] Estonia [ ] Finland
 [ ] France [ ] Germany [ ] Greece [ ] Ireland [ ] Italy [ ] Lithuania [ ] Netherlands [ ] Northern Ireland [ ] Norway [ ] Poland
 [ ] Portugal [ ] Russian Federation [ ] Scotland [ ] Slovakia [ ] Slovenia [ ] Spain [ ] Sweden [ ] Switzerland [ ] Ukraine [ ] Wales

 Mode
 [ ] Default [ ] DAFC

SSR Data Analysis with Poppr

Data Summary  Genotype Freq  Min. Spanning Tree  Phylogenetic Tree  PCA  MLG INFO  Download  Parallel C

No of multilocus genotypes: 306
No of individuals: 1412
No of codominant loci: 12

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<th>Allele</th>
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<th>Evenness</th>
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Populations can be removed from further analysis. Activate below.

Activate subsetting
[ ] Yes
[ ] No

Choose pop to be removed

Select Pop Structure for subsetting
[ ] genotypes [ ] Country [ ] Year

Collapse MLGs
[ ] Yes [ ] No
2013-15 Genetic diversity - PCA

- Distinct clusters of major clones
- Minor variation within clones
- Greater genetic diversity of ‘Other’ genotypes apparent
- PCA an imperfect tool as the cloud of data obscures some points
• 2013-2015 data after removing the ‘Other’ category
• Distinction **between** most clones is clear
• Minor genetic variation **within** clones apparent
• Example below shows minor differences in 1 to 3 SSR loci of genotype EU_13_A2
• Such variation useful to track variants of clonal lineages

<table>
<thead>
<tr>
<th>SSR locus</th>
<th>Pi02</th>
<th>Pi4B</th>
<th>G11</th>
<th>Pi04</th>
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</table>
SSR diversity minimum spanning tree

- EU 2014 – 1552 isolates

- Node size proportional to isolate no.
- Edge thickness and colour are proportional to Bruvo's distance.
  Edge lengths are arbitrary.
- Limited area so edges overlap
Genetic diversity - MSN

- Distances **within** variants of main clones short
- Distances **between** main clones long (arrows)
Data kindly provided by CIP

Application in Latin America
Benefits of data entry

- Standardised protocols and data format for comparisons between countries/regions
- Availability of other data-sets (once agreed by data owners)
- State-of-art analysis tools
- Production of publication quality figures

- Agreement needed on data sharing and data-usage
- Shared experiences of managing globally distributed strains
- Advance warning of new threats (Biosecurity in seed trade)
IPM Blight 2.0 – EU project

ERA-NET IPM call

- France
- Denmark
- Estonia
- Norway
- Scotland

- Started Apr-16
Conclusions & future plans

- New insights on pathogen diversity
- Dominant clones across European crops - we share management challenges
- Much primary inoculum asexual and locally propagated. Better management of this needed
- Other populations highly diverse & ephemeral as a result of sexual oospore formation
- High genetic diversity increases the risk of blight management problems

- Phenotypic traits of existing and novel genotypes examined in a 2016 new ERA-NET project (IPMBlight2.0)
- Discussions with other networks ongoing - same model to study population and share data globally (AsiaBlight, USABlight, TizonBlight)
- Co-operation is critical
Thanks

ADAMA
Agrifirm
Agriphar
BASF
Bayer CropScience AG
Bayerische Landesanstalt für Landwirtschaft
Belchim Crop Protection
Centre Wallon de Recherches Agronomiques
Certis
Cheminova
CZAV
Dupont de Nemours
Emsland Group
Germicopa SAS
HZPC Holland B.V.
AHDB Potatoes
Neiker
Nordisk Alkali
PCA
Profytodsd
Swedish University of Agricultural Sciences
Syngenta Agro GmbH
Agricultural Institute of Slovenia
AFBI, Northern Ireland
Estonian University of Life Sciences